A study on some aspects of the pathogenicity, diagnosis and control of gastrointestinal nematodes in deer

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Abstract

The most important parasites in farmed red deer are *Dictyocaulus eckerti* and gastrointestinal nematodes (GIN). The overall aim of these studies was to develop an understanding about GIN parasites in red deer, including their pathogenicity, diagnosis, control and the risk of cross-infection with cattle/sheep. To understand the pathogenicity of GIN, young deer were trickle infected with a mixed culture of deer-origin infective larvae (L3). The infection comprised 40% *Ostertagia*-type and 53% *Oesophagostomum* spp. L3. As a result of the high proportion of *Oesophagostomum* spp. L3, the animals were clinically affected with large intestinal lesions and it was not possible to investigate the effect of *Ostertagia*-type parasites. *Oesophagostomum sikae* was recognised in New Zealand for the first time in this study. A national survey of the prevalence of different GIN in deer utilised PCR-based methodology. From each of 59 deer farms around New Zealand faeces from an average of 19 deer/farm were cultured and 24 infective larvae were randomly selected and identified. The order of prevalence from high to low was *Oesophagostomum. venulosum* > *Spiculopteragia asymmetrica* > *S. spiculoptera* > *Ostertagia leptospicularis*. This illustrated the importance of abomasal nematodes in the subfamily Ostertaginae. A study was conducted to determine the ability of sheep GIN to establish in deer. The highest establishment rates were *Haemonchus contortus* (10.5%), *Trichostrongylus axei* (12.2%) and *O. venulosum* (5.8%). However, these were all lower than in sheep. The effectiveness of cross-grazing system between deer and sheep (DS) or cattle (DC) compared to deer grazing alone (DD) was undertaken as a replicated study at two locations over two years. The key outcomes were that DC needed fewer anthelmintic treatments and still had higher live-weight than other groups. The DD group received more treatments and still had highest nematode counts for *Ostertagia*-type nematodes and *Dictyocaulus*. The DS group received a similar number of treatments to DD and had the highest burdens of *T. axei*. Cross-grazing offers advantages which varied between DC and DS with regards the level of control of GIN, however, both were effective in controlling lungworm infection. Deer in all groups still required anthelmintic treatment to maintain growth rates.

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Table of contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Description
°C	Degree Celsius
µm	Micrometre
AT	Anthelmintic Treatment
BZ	Benzimidazoles
CI	Confidence Interval
CT	Condense Tannins
D	Deer only
DC	Deer and Cattle
DCS	Deer, Cattle and Sheep
DD	Deer only
DS	Deer and Sheep
FEC	Faecal Egg Count
FLC	Faecal Larval Count
g	Gram
GIN	Gastrointestinal Nematodes
HD	High Dose group
InvAgR	Invermay AgReseach
ITS	Internal Transcriber Spacer
kg	Kilogram
l	Litre
L1	First Stage Larvae
L3	Third Stage Larvae
LD	Low Dose group
LrD	Lower Dose group
LSM	Least Squares Means
LWG	Liveweight Gain
MD	Medium Dose group
mg	Milligram
ml	Millilitre
PCR	Polymerase Chain Reaction
PNMassey	Palmerston North Massey Deer Unit
qPCR	Real time PCR
rDNA	Nuclear Ribosomal DNA
SAS	Statistical Analysis System
SE	Standard error of the mean
SP	Deer only Supressive treated
Taq	Thermus Aquaticus
VFI	Voluntary Feed Intake
WAAVP	World Association for the Advancement of Veterinary Parasitology

Preamble

Before this thesis was planned the impact of infections with gastrointestinal nematodes (GIN) on young deer was poorly understood and the ability to diagnose them was equally poor except for estimating faecal egg counts. Earlier studies evaluated the impact of internal parasitism in a field environment including demonstrating subclinical losses, the value of some markers as diagnostic indicators for the need for anthelmintic treatment use, and identified the species of internal parasites present. However, those studies evaluated the combined impact of both lungworm together with GIN and were not able to partition the effects to either category of parasite. Another important recent issue was the identification of the apparent anthelmintic resistance in the *Ostertagia*-type parasites. This has resulted in more attention being focused on the GIN and has also increased their importance in the field. Therefore, many aspect of GIN needed to be further investigated.

My initial involvement starts in May 2009 but was interrupted by maternity leave from May 2010 until December 2010. It was still possible to conduct some research over this maternity leave or to at least collect samples for later analysis as indicated below.

In 2009 research for this thesis commenced and the funding was provided by DEEResearch New Zealand Ltd and AgResearch Ltd. The initial pathogenicity study started back in April 2009. The aim of that study was to understand the pathogenicity of gastrointestinal parasites in housed weaner deer. My involvement in this study commenced after the experiment was initiated but I was responsible for analysing the samples and assessing the results. A feature of this initial study was the development of severe clinical signs in many of the deer, especially those receiving the highest dose rate of larvae. This necessitated the early termination of the study and implementation of a follow-up pathogenicity study (Chapter 3) that investigated the impact of artificial infection with a lower dose of deer-origin larvae. I was fully involved with the design, day-to-day running of this experiment as well as analysis of all samples.

In parallel with the pathogenicity studies in 2009, opportunistic use was made of faecal samples collected during May 2009 and September 2010, primarily for a multi-species study on the epidemiology of Johne's disease executed by Cristobal Verdugo and collaborators. Once these researchers had taken their faecal samples the remaining faeces became available for us to investigate the occurrence of different GIN species in red deer in New Zealand. The sampling regime was designed for the Johne's disease study but also suited our purposes. The larvae were stored at 10°C until selected for DNA extraction. After the individual DNA extraction, the larvae were frozen until required for later PCR identification.

The cross-grazing field study to determine the value of an organised cross-grazing system between deer and sheep or cattle to assist with the control of deer internal parasites, was replicated at two locations and over two years, 2012-2013. I am indebted to Dr Mackintosh of AgResearch, Invermay for running one of the replicates on the Deer Research Unit at Invermay. We discussed the overall design of the experiment and were aware of the day-to-day events occurring at Invermay during this study. However, neither I nor my Massey University supervisors ever visited the Invermay site during this study. Most of the faecal samples and gastrointestinal samples for parasitology were sent to our laboratory for analysis. The preparation of paddocks in the two farms prior to the starting of the cross-grazing trial, started on December 2011 for the first year and on December 2012 for the second year. The animals of the trial started grazing and rotating on the paddocks of the study when they were weaned, this happened at the beginning of March in 2012 and at the end of February in 2013. The experimental phase lasted until June both years.

In parallel with the cross-grazing study, in April 2012, a cross-infection study was implemented to determine the establishment rate of sheep GIN in young deer compared with sheep of the same age to help understand the potential risks associated with cross-grazing and susceptibility of deer to sheep GIN. The deer and the sheep were infected (3 May 2012) one time with the infective larvae by stomach tube and four weeks after infection all animals were euthanized (31 May 2012). A similar study investigating the infectivity of cattle GIN for deer was conducted by another post-graduate student S.J. ten Doesschate but with which I was also involved although it isn't formally part of my own PhD studies.

The experiments in this thesis were approved by the Massey Animal Ethics Committee (MUAEC 12/09, MUAEC 12/10, MUAEC 09/28) or the AgResearch Animal Ethics Committee.

Chapter 5 was submitted for journal publication and Chapter 2, 4 and 6 will be submitted for journal publication. The chapters will be modified for the required publication style format.

Chapter 1 literature review

1.1 Deer farming

1.1.1 Deer species and production around the world

The Cervidae family includes around 40 species and 200 subspecies. The Cervinae subfamily includes the most commonly farmed species. Some of them are only extensively farmed, including hog deer (*Axis porcinus*), sambar deer (*Cervus unicolor*), rusa deer (*Rusa timorensis*), axis deer (*Axis axis*), Père David's deer (*Elaphurus davidianus*), white-lipped deer (*Cervus albirostris*) whilst others are intensively farmed including sika deer (*Cervus nippon*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus)* (Haigh 1995). The most common species to be intensively farmed is *C. elaphus* and includes around 22 sub-species (Ludt *et al.* 2004). Wapiti, a native species of Canada is generally considered to be a separate species (*C. canadensis*) but others consider it a sub-species of *C. elaphus* with which it interbreeds (Kuznetsova *et al.* 2012).

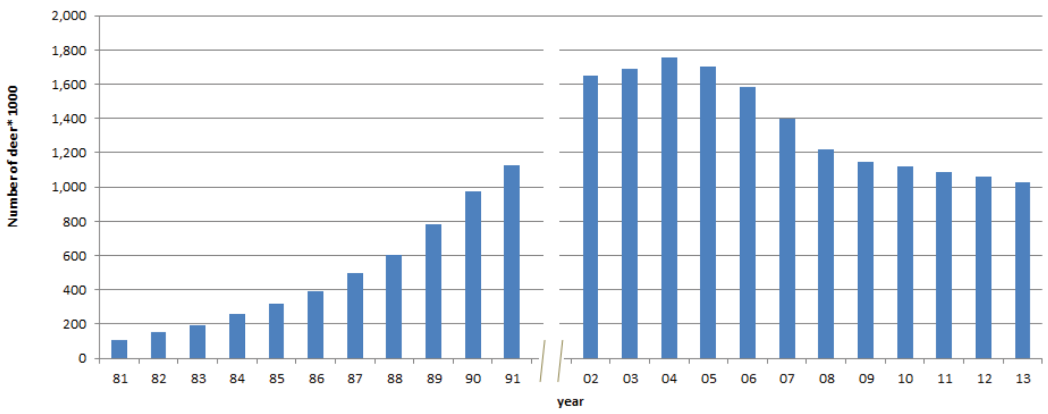
Modern deer farming began in New Zealand in the late 1960's and in 2013/14 there were about 2,800 deer farms with 498,000 female deer mated. The absolute majority of these were red deer (StatisticsNewZealand 2013). Deer farming dates back about 3,000 years with the earliest reports from China, where at the present time the majority of deer farms are small or medium scale (Zheng *et al.* 2002) and the industry is mainly focused on velvet production. In 2002 there were more than 500,000 farmed deer in China, including sika deer (350,000), red deer (150,000), white-lipped deer (2,000) and sambar deer (1,500). This compares with approximately 10,000 deer farms in Europe and 1,200 in Australia (Chardonnet *et al.* 2002). Worldwide it was estimated there were around 5 million farmed and ranched deer in 35 countries in 2002 (Chardonnet *et al.* 2002).

1.1.2 Population and distribution in New Zealand

Between 1861 and 1923 seven species of deer were introduced into New Zealand and were rapidly established in the wild. A total of 1000 red deer were liberated between 1861-1923 mainly from England and Scotland, 130 fallow deer from 1864-1910, 26 sambar deer in 1875 and 26 during 1914-1921, 18 wapiti in 1905, and small numbers of sika deer, white-tailed deer (*Odocoileus virginianus borealis*) and rusa deer (Challies 1985).

The successful establishment of red deer in New Zealand has been attributed to a variety of factors including: moderate temperate climate, absence of food competitors and predators, as well as countless natural protected areas in the forests (Challies 1985) . Commercial venison production started around 1958-1959 with animals shot in the wild being exported to Europe, particularly to the Federal Republic of Germany. However, given this intensive hunting, there was a huge reduction (75-95%) in deer numbers. To meet the steady and high demand of venison in Europe, deer farming became legal in 1969 following changes to the Animal Act in 1967. From that time the number of deer farms gradually increased, growing slowly from 1970 till 1983 when there were 240,000 animals, mainly red deer, being farmed. Many of these (60,000) were originally captured from the wild (Challies 1985). The number of farmed deer peaked in 2004 with 1.757 million and has been steadily declining since then, with a population of 1,028 million in 2013 **(StatisticsNewZealand 2013)**.

Figure 0.1 The New Zealand farmed deer population 1981-2013.



Interestingly the number of farmed deer varies between the North and South Islands. The farmed population in the North Island increased steadily until 1991 when it stabilized until 2004. After that time the population has gradually dwindled until 2013, the last year of available data, when it was 321,000. In contrast, in the South Island, the population increased until 2005 reaching 1,153,000 and thereafter has declined only slightly each year until 2013 when it was 1,028,000. In 2013 the majority (66%) of farmed deer were in the Canterbury, Southland and Otago regions. The number of deer in different regions in 2013 were: Canterbury 288,000; Southland 221,000; Otago 169,000; Waikato 79,000; Manawatu-Wanganui 76,000; and Hawkes Bay 69,000 deer (StatisticsNewZealand 2013).

1.1.3 Venison "deer meat".

Originally the term "venison" was utilized to refer only to the meat of an animal killed by a hunter. More recently it is used to describe meat from a deer, regardless of whether it is hunted or reared on a farm and killed in a slaughterhouse. For the purpose of this literature review, venison will be used to describe all deer meat.

Internationally, most wild venison comes from Northern Europe, North America, and Russia and most farmed venison comes from New Zealand. In 2013, thirteen thousand tonnes of venison were exported with a free-on-board value of $163,722,087 in New Zealand. The greatest consumers of venison (Shadbolt *et al.* 2008) are Scandinavians, Europeans and Russians, because traditionally they consumed game meat during the hunting season (October-December). Thus for New Zealand, it is important to have finished product by October to be shipped to meet this demand. This then means that maximising early growth is important for New Zealand deer farmers.

1.1.4 Velvet Antler

Antlers are secondary sexual characteristics of male red deer. They are cranial osseous appendages first developed in puberty with cyclical replacements every year. The seasonal development of the antlers is stimulated by light cycles and sex hormones.

Velvet antler refers to the whole antler in a pre-calcified stage of growth. In 2 year old stags it has to be removed after 40-50 days of growth, and in older animals after 60-75 days (Moore *et al.* 1985). Velvet antler is a valuable material in traditional Chinese medicine. New Zealand produces the most velvet internationally with Asia the most common destination, mostly Korea. To date there is no direct evidence that body condition in adults influences antler growth. However, a study on antler growth in young white-tail deer observed that food (principally protein) restriction and/or mineral deficiency can affect antler growth (French *et al.* 1956).

1.1.5 Deer seasonality

Deer respond to circadian and circannual variation. The pineal gland responds to decreasing day length increasing the production of the hormone melatonin. Different concentrations of this hormone will influence: feeding patterns; variation in antler growth; live-weight gain; and changes in the reproductive cycle (Simpson *et al.* 1984; Sibbald and Milne 1993; Stafford *et al.* 1993; Asher 2011; Asher *et al.* 2011a; Asher *et al.* 2011b)

1.1.5.1 Feeding

During longer days, food intake increases which is reflected in higher live-weight gain with the opposite during shorter days, when deer consume less and gain less weight. Therefore, farmers aim to match the pasture growth curve with deer requirements. Farmers appreciate the need to maximise growth during longer days and this can be achieved by either matching the deer demand to the feed supply or the supply to the demand.

1.1.5.2 Reproduction

Red deer have a seasonal reproductive pattern triggered by melatonin production. Females are short day breeders with conception occurring during autumn and subsequent calving occurring during summer. In New Zealand calving usually commences in November extending through to December. Consequently, weaning occurs in late February, early March. Puberty is reached when females reach a target live weight together with the reduction of the daylight hours in autumn. On average they reach puberty in their second autumn of life (16 months) (Asher 2011; Asher *et al.* 2011a; Asher *et al.* 2011b)

1.2 Nematodes

1.2.1 Introduction

Parasitology is the science which studies parasites (Reinecke 1983). Parasites, from the Greek “situated beside”, are “organisms that live on or within some other living organism, which is known as the host” (Reinecke 1983).

Parasitic worms are typically from the helminth taxa. The parasitic helminths of veterinary importance are contained by the Phyla Nematoda (roundworms), Platyhelminthes (flatworms, tape worms and flukes) and Acanthocephala (thornyheaded worms) (Bowman 2014).

In domestic animals, nematodes are a major cause of unhealthy herds and losses in production (Sykes 1994; Corwin 1997; Zajac 2006). The impact of nematodes in the host is affected by the species, the target organ, their life cycle and their strategy to establish in their host. They are also affected by the host's immune system, which in turn is influenced by age and size of the animal (Greer *et al.* 2009), species or breed, hormones, previous infection, management of the farm including nutrition, grazing management, anthelmintic utilization (Sykes 1994; Coop and Kyriazakis 2001). Understanding the epidemiology of nematodes together with good management usually results in few clinical cases of parasitism. However, the growing development of anthelmintic resistant strains has resulted in a reassessment and this is recognized as one of the greatest menaces to the industry (Waller 1997).

1.2.2 Structure

Nematodes are cylindrical, slender and active worms usually narrow at both ends, presenting a body covered by a tough outer cuticle. The phylum includes parasitic species of animals and plants (accidental or obligatory parasitism). It also includes free living nematodes of water and soil.

In general nematodes are dioecious with sexual dimorphisms, where the female is bigger than the male. The female reproductive apparatus includes an ovary, oviduct, uterus, vagina and vulva. The male possesses a testis (generally one) and vas deferens leading to an ejaculatory duct which opens with the digestive tract in the cloaca. Males, especially in the Superfamily Trichostrongyloidea, have accessory sexual organs including, spicules (generally a pair) and a gubernaculum. These chitinous structures are important during copulation. Because these structures are different between taxa, both are important in the morphological identification to species level.

Depending on the genus or species the cuticle can be modified into leaf crowns, papillae, cervical and caudal alae, cephalic and cervical vesicles as well as a copulatory bursa (Figure 0.3). The presence or absence of these, together with their particular morphology, helps in the identification of the different taxa. Some species e.g. the *Ostertagia* spp., may also develop structural polymorphism, having minor and major morph types of the same species with differences between them in the shape of the spicules and the genital cone.

Figure 0.2. Cephalic region – *Oesophagostomum venulosum*.

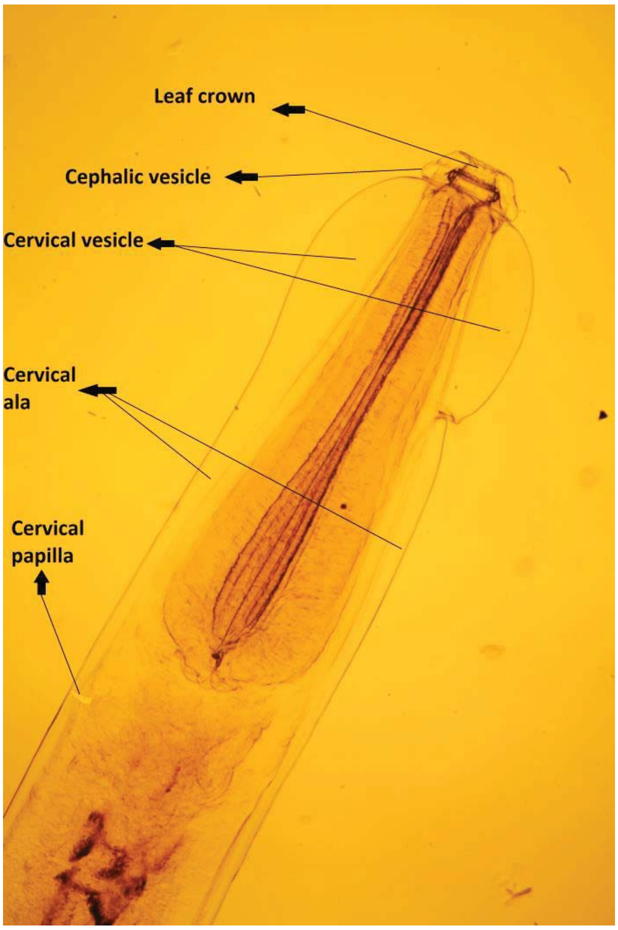
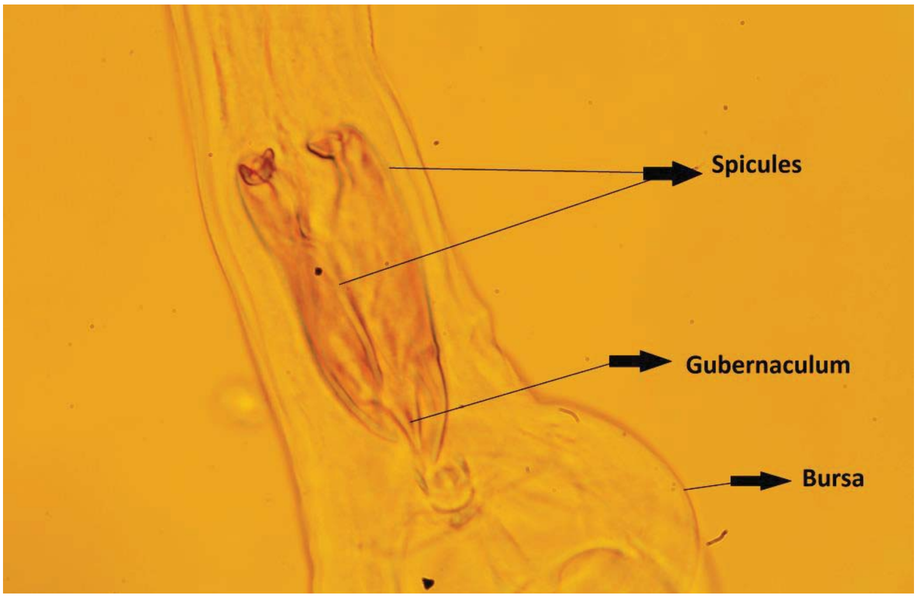


Figure 0.3. Male spicules and bursa – *Trichostrongylus askivali*



1.2.3 Life Cycle

The parasitic nematodes of major economic importance in ruminants belong to the Order Strongylida and particularly to the Superfamilies Trichostrongyloidea and Strongyloidea. They have a direct life cycle, where the egg or first stage larvae (L1) leave the host's body in the faeces. The L1 hatches from the egg and feeds on bacteria prior to moulting to the second and then the third larval stage (L3). The development from egg to L3 is very dependent on environmental conditions, especially temperature and moisture. The response to temperature behaves as a growing degree-day model which requires a number of degree-days above a minimum for the larvae to develop. In the last moult strongylid larvae retain the cuticle of the second larval stage. Thus the L3 is said to be ensheathed and this is the infective stage for the definitive host. The retained cuticle around the L3 provides additional protection improving the survival of this stage in the environment. The ensheathed L3 is, however, unable to feed and relies on stored metabolites. The rate that larvae use up these stored metabolites and hence survive is dependent on temperature. Desiccation is another important cause of death. However, some are able to tolerate extreme temperatures (high and low) and desiccation. (Wertejuk 1959; Todd *et al.* 1976; Lee 2002; Lettini and Sukhdeo 2006).

The host ingests the L3 whilst grazing and the larvae then proceed through the L4 and adult stage in their preferred location. In the final host, the time from infection to the production of larvae or eggs is the prepatent period and for trichostrongyloids is usually between 2-4 weeks. The diets of most trichostrongyloid species are based on gastrointestinal secretions and mucus, with the exception of *Haemonchus contortus* which feeds on blood (Pomroy 1997). They also have the ability to enter an inhibited phase where development is suspended at a larval stage within the animal, influencing the epidemiology of the parasite.

1.3 Nematodes in Deer

In New Zealand, parasites are an important economic and clinical problem (Audigé *et al.* 1998) and it has been acknowledged as a problem since the commencement of deer farming. The most important parasites in farmed deer are the lungworm *Dictyocaulus eckerti* and the gastrointestinal nematodes (GIN). The majority of the important GIN are located in the abomasum, including *Spiculopteragia asymmetrica, Spiculopteragia spiculoptera* (=*Spiculopteragia boehmi*), *Ostertagia leptospicularis (=Ostertagia crimensis)* and some *Trichostrongylus* species. Farmed deer in New Zealand have only small burdens of small intestinal species but in the large intestine *Oesophagostomum venulosum* is common. Collectively from around the world there are limited studies on the parasites of any deer species and red deer in particular.

1.3.1 Dictyocaulus spp.

In ruminants lungworms in the genus *Dictyocaulus* induce respiratory disease and in deer in New Zealand lungworm has been recognized as the most important parasite. It can cause severe disease in naïve animals particularly in deer calves during their first autumn (Charleston 1980; Mason 1985a). Two species have been described in deer, *Dictyocaulus eckerti*, whose preferred host are deer and *Dictyocaulus viviparus* whose preferred host are cattle (Johnson *et al.* 2003b). Historically there has been considerable confusion as to the identity and host specificity regarding these two species. In the early 20th century Railliet and Henry (1907; cited by Skryabin et al. 1954) named the species from *Cervus elaphus* as *Dictyocaulus noerneri* but the authors gave only a brief description concentrating on the length of the spicules. A subsequent mention by French authors described *D. noerneri* as being commonly found in deer (Skryabin *et al.* 1954). Chapin (1925; cited by Skryabin el al. 1954) described *Dictyocaulus hadweni* from *Bison bison* and wapiti but again did not give a detailed morphological description. In 1931 Skryabin named and described *D. eckerti* (Skryabin *et al.* 1954) from the lungs of a reindeer, but it was not until 1995 that *D. eckerti* from a fallow deer and *D. viviparus* from cattle were recognized as being genetically different species (Epe *et al.* 1995). At this point in time *D. eckerti* is the generally accepted name for the *Dictyocaulus* species which parasitizes several species of deer. *D. eckerti* was recognised as the dominant species in red deer in New Zealand in the early 21st century (Johnson *et al.* 2001a; Johnson *et al.* 2001b). The same authors later showed that red deer can be infected with both *D. eckerti* and to a lesser extent with *D. viviparus* but *D. eckerti* does not successfully establish in cattle (Johnson *et al.* 2003b). The other important *Dictyocaulus* species in farmed ruminants is *Dictyocaulus filaria,* which has sheep and goats as its preferred hosts, but there are no reports of red deer being infected with it. Taxonomic studies are important to understand the epidemiology of each species of lungworm, especially when cattle, sheep or deer co/cross-graze the same area (Wilson 2002) and also when wild deer are able to move many kilometres carrying and spreading nematodes in a region (Chintoan-Uta *et al.* 2014).

1.3.1.1 Description

Adults are thin thread-like or filiform white worms with females larger than the males. *Dictyocaulus eckerti* males measure 1.9-4.0 cm and females 3.1-6.5 cm (Skryabin *et al.* 1954) and *D. viviparus* males 4.0-5.5 cm and females 6.0-8.0 cm. *D. eckerti* can be differentiated from *D. viviparus* on the size and shape of the buccal ring, which for *D. eckerti* is oval and for *D. viviparus* is circular (Johnson *et al.* 2001a). *D. eckerti* has also been described as having longer spicules with a different shape of the end of the dorsal ray and the presence of cervical papillae and a buccal capsule (Skryabin *et al.* 1954). However morphological characters are not totally reliable, especially when *Dictyocaulus* may be in a different host or when the size of the nematode is influenced by the host (Divina *et al.* 2000) making the morphological differentiation of *D. eckerti* from *D. viviparus* difficult.

1.3.1.2 Life cycle and epidemiology.

Dictyocaulus spp. have a direct cycle. The adult worms live in the larger airways and lay eggs containing fully developed L1. These hatch within the lungs and the L1 subsequently move up the trachea to the pharynx where they are swallowed to the gastrointestinal tract and then are eliminated through faeces. The L1 moult to L2 and retain the cuticle of the L1 indicating L2 do not feed. These then moult to the L3 and initially at least, are contained within the retained cuticle of both the L1 and L2. The cycle continues when the L1 in the environment become infective L3 and another deer swallows it. The L3 inside the body penetrate the intestinal mucosa after exsheathment and start travelling principally via the lymphatic circulation through the mesenteric lymph nodes reaching the lung capillaries and breaking into the alveoli. This may occur after only one week from ingestion of the L3. The last moult of the larvae takes place in the bronchioles and a few days later the young adults mature in the bronchi (Mason 1985b; Taylor *et al.* 2007). The minimum prepatent period in red deer for *D. eckerti* is 23 days and for *D. viviparus* 22 days (Johnson *et al.* 2003b).

Disease is seen frequently in regions with high rainfall or on farms with irrigation (Breeze 1985). It is believed that the main source of infection is fawns over two months old (Mason 1985b). There are only limited studies on the preferred conditions for the development and survival of infective *D. viviparus* larvae and even less for *D. eckerti*. By comparison with other trichostrongyloids their free living stages are more susceptible to various environmental effects such as dryness and high temperatures. They generally do not survive for long periods (Rose 1956). However, some research has shown that under certain conditions of low temperatures some L3 of *D. viviparus* are capable of survival overwinter (Gupta and Gibbs 1970; Strube *et al.* 2007; Laabs *et al.* 2012), though this hasn't been yet proved for *D. eckerti.*

1.3.1.3 Pathogenicity, clinical signs

Clinical disease is common in young animals before 12 months of age, especially where control on-farm is poor. There are only a few descriptions of the pathogenesis of lungworm in deer. In cattle in Europe, the disease cause by *D. viviparus* is often seen during their first season on grass and pathogenesis has been described in three stages.

1) The prepatent stage extends from day 7-25. At this time the main signs are a cough and increase in the respiratory frequency. The severity of the disease depends on the number of larvae ingested and/or the numbers that reach the lungs. The damage from larvae penetrating the alveoli then entering the bronchioli and finally when the adult reaches the larger airways of the bronchi, causes alveolitis, bronchiolitis and bronchitis, respectively (Breeze 1985; Panuska 2006; Taylor *et al.* 2007). The prepatent phase is generally considered to be clinically significant.

2) The patent stage extends from day 25-60 (Breeze 1985; Panuska 2006; Taylor *et al.* 2007). Over this period the adults are in the larger airways laying eggs. It is a very important stage epidemiologically and also very important clinically. Coughing may become more severe with a further increase in respiratory frequency, pyrexia, decreased food intake, and weight loss (Breeze 1985; Panuska 2006; Taylor *et al.* 2007). At this stage, the excess mucus production may be blocking the lumen of the bronchi and smaller airways leading to emphysema and pulmonary consolidation. It is compounded by the aspiration of L1 and eggs into the smaller airways and alveoli (Breeze 1985; Panuska 2006; Taylor *et al.* 2007).
3) The recovery stage extends from day 55-90 (Breeze 1985; Taylor *et al.* 2007) and is when the animals start to recover.

In deer, depending on the level of infection of *D. eckerti*, the signs can also go from loss of production to death. It appears that the pathogenicity in deer is slightly different from cattle. The airways seem to have less exudate and fewer consolidated areas. Even in fatal cases, the lungs show minimal macroscopic damage with most signs attributed to the obstruction of the trachea and bronchi by adult lungworms (Charleston 1980). Deer do not generally develop the pronounced coughing at rest described in cattle, making diagnosis based on clinical signs unreliable. Some of the non-specific signs in deer are reduced voluntary food intake with consequent loss of condition and reduced live-weight gain (Corrigall 1985).

1.3.2 Varestrongylus sagittatus.

This protostrongylid (Nematoda: Metastrongyloidea) lungworm was first identified in red deer in New Zealand in the 1990s (Mason 1994). Unlike *Dictyocaulus* spp. where the adults are located in the airways, *Varestrongylus* spp. adults are located in nodules in the lung tissue and it is difficult to recover intact nematodes from lung washes. It was probably in New Zealand well before Mason's initial report but had gone unnoticed. As with other protostrongylids the L1 is eliminated in the faeces and its life cycle requires gastropod molluscs as intermediate hosts for the development of the L1 to L3. The L1 of *Varestrongylus* has a spiny tail (Mason 1995) and it is morphologically similar to *Elaphostrongylus* spp. and *Muellerius* spp , protostrongylids. The spiny tail allows it to be differentiated from the L1 of *Dictyocaulus* spp. which does not have this feature.

1.3.3 Gastrointestinal nematodes

Gastrointestinal nematodes have always been recognised as a potential issue for red deer (Mason 1977; Watson and Charleston 1985c; Audigé *et al.* 1998). However, from the early stages of deer farming in New Zealand, the emphasis was on controlling lungworms with anthelmintics and it was presumed this would also control gastrointestinal parasites. Nevertheless, high burdens of GIN were reported in farmed deer in the seventies (Mason 1977). Studies on the benefits of anthelmintic use found significant differences in live-weight gain between treated and untreated deer but as these animals were on pasture it was not possible to partition the effects of lungworm from those of GIN (Wagner and Mackintosh 1993a; Waldrup and Mackintosh 1993; Waldrup *et al.* 1994). The only experimental studies to date have also combined both GIN and lungworm infections (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b)

The most relevant GIN (Table 0.1) are located in the abomasum and are deer-specific nematodes of the Sub-family Ostertagiinae (=*Ostertagia*-type), including three related species, *S. asymmetrica, S. spiculoptera* and *O. leptospicularis.* Naming of these species has been somewhat confusing since their original descriptions but now seems generally accepted as indicated. Each of these has both major and minor morph types. *Trichostrongylus axei* has also been commonly reported from the abomasum of deer together with *Trichostrongylus askivali* (McKenna 2009c). The latter is a deer-specific nematode. *Haemonchus contortus* and *Teladorsagia circumcincta,* both of which are principally sheep species, have also been reported from red deer (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b; Manfredi *et al.* 2007). *Ostertagia ostertagi,* which has cattle as its preferred host has also been reported in red deer overseas but not in New Zealand (Pursglov *et al.* 1974; Conti and Howerth 1987; Drozdz *et al.* 2002).

Small burdens of some other cattle and sheep nematodes have been reported in red deer in New Zealand including *Trichostrongylus vitrinus, Trichostrongylus colubriformis, Cooperia pectinata, Cooperia curticei, Cooperia oncophora, Cooperia punctata* (McKenna 2009a).

Oesophagostomum venulosum is commonly found in the large intestine of red deer in New Zealand. Its preferred hosts are sheep and goats (Hoskin *et al.* 2000b; McKenna 2009a). Reports of small to medium burdens of *Oesophagostomum radiatum,* which has cattle as its preferred host, were reported in adult red deer in New Zealand (McKenna 1999) and in fallow deer in Germany (Barth and Matzke 1984). *Oesophagostomum sikae* has also been recognised in several species of deer including sika deer (Cameron and Parnell 1933; in Popova 1965), red deer and roe deer (Patrelle *et al*. 2014). This species is morphologically similar to *O. radiatum* and has recently been recognised in New Zealand (see Chapter 2). It is likely that earlier reports of *O radiatum* in deer in New Zealand have misidentified *O. sikae.* There is also one report of *Trichuris ovis* (Andrews 1973) in red deer but no more in recent years.

1.3.3.1 *Ostertagia*-type nematodes

1.3.3.1.1 Description

There are three common *Ostertagia*-type species in deer: *S. spiculoptera* (Gushanskaya, 1931 cited by Skryabin 1954) and its minor morph *S. mathevossiani* (Ruchlyadev, 1948 cited by Skryabin 1954), *S. asymmetrica* (Ware 1925) and it minor morph type *S. quadrispiculata* (Jansen 1958), and *O. leptospicularis* (Assadov, 1953 cited by Skryabin 1954) and its minor morph *O. kolchida* (Popova, 1937 cited by Skryabin 1954). The minor morph types are by definition less common than the major morph type. The proportion varies but is commonly ≤ 10%.

It is common to find all three Ostertagia-type species (*S. spiculoptera, S. asymmetrica* and *O. leptospicularis*) in the abomasum of red deer in New Zealand.

Table 0.1 Description of some relevant abomasal GIN of deer in New Zealand.

species	host reported	Synonym	Morph-type	original description	Female	male	spicules length	description of species in
Spiculopteragia spiculoptera	Cervidae: including red deer, roe deer, sika deer, white-tailed deer, sambar deer, fallow deer, reindeer and moose	*Ostertagia böhmi* (Gebauer 1932) *Ostertagia spiculoptera*	**Major** ***spiculoptera***	Gushanskaya, 1931	6 - 7.5 mm (Andrews 1969) 6.9 - 7.9 mm (Pato 2012)	O. bohmi 6-7 mm (Gebauer 1932) 4.5- 7 mm (Andrews 1969) 6.5-7.4 mm (x̄=6.8) (Umur *et al.* 2011) 8.89 mm (*Gushanskaya, 1931)	160-175 (Gebauer 1932) 140-200 µm (Andrews 1969) 155-192 µm (x̄=174 µm) (Umur *et al.* 2011) 199 µm long (*Gushanskaya, 1931)	chamois (Gebauer 1932) wild deer (not specifically state (Andrews 1969) roe (Umur *et al.* 2011) sheep (*Gushanskaya, 1931)
	Bovidae: chamois, tahr, mouflon, sheep, goats and cattle. Camelildae: 1 report in llama	*Rinadia mathevossiani*	**Minor** ***mathevossiani***	Ruchlyadev, 1948		6.6-7.9 mm (x̄=7.4) (Umur *et al.* 2011) 7.2 mm (* Ruchlyadev, 1948) 8- 8.8 mm (Pato 2012)	188-222 µm (x̄=202 µm) (Umur *et al.* 2011) smallest= 189 µm largest=200µm (* Ruchlyadev, 1948) 183-204 µm (Pato 2012)	roe (Umur *et al.* . 2011,* Ruchlyadev, 1948)
Spiculopteragia asymmetrica	Cervidae: including red deer, roe deer, sika deer, white-tailed deer, fallow deer, reindeer, and moose.		**Major** ***asymmetrica***	Ware, 1925	6 mm (Ware 1925)	x̄= 5. up to 6.5 mm (Ware, 1925)	200 µm (one w/trumpet-like ramification)	fallow deer (Ware, 1925)
	Bovidae: chamois, sheep. Camelildae: 1 report in llama	*Apteragia quadrispiculata* (Jansen 1958)	**Minor** ***quadrispiculata***	Jansen, 1958		3,8 a 7,0 mm (Jansen, 1958)	smallest= 182-222 µm largest=186-226µm (Jansen, 1958)	fallow deer (Jansen, 1958)
Ostertagia leptospicularis	Cervidae: red deer, fallow deer, roe deer, elk, moose, sika deer and caribou.		**Major** ***leptospicularis***	*Assadov, 1953	8-9 mm (Taylor *et al.* . 2007)	6.9-8.3 mm (* Assasov, 1953) 5- 6.5 mm long (Andrews 1969)	203-218 µm (* Assadov, 1953) 160- 200 µm (Andrews 1969)	roe deer (* Assadov, 1953)
	Bovidae: cattle, chamois, sheep.		**Minor** ***kolchida***	Popova, 1937	7-7.6 mm (* Popova, 1937)	6.2-6.8 mm (* Popova, 1937)	140-150 µm (* Popova, 1937)	cattle
Teladorsagia	Cervidae: red deer, fallow deer, roe deer, caribou, elk,	*Ostertagia circumcincta,*	**Major**	Stadelmann, 1894	12.5-13.5 mm (* Petrov and	9.8-10.64 mm (* Petrov and Shakhovtseva,	400-420 µm (* Petrov and	sheep (* Petrov and Shakhovtseva, 1926)

species	host reported	Synonym	Morph-type	original description	Female	male	spicules length	description of species in
circumcicta	mule deer, white-tailed deer, muskox.	*Strongylus circumcinctus, S. vicarious, S.cervicornis, S. instabilis or Ostertagia turkestanica*	***Circumcincta***		Shakhovtseva, 1926)	1926)	Shakhovtseva, 1926)	
	Bovidae: cattle, sheep, goat, mouflon, antelope.	*Ostertagia pinnata* (Daubney, 1933)	**Minor** ***trifurcata***	Ransom, 1907	10 mm (* Travassos, 1921)	7-8.5 mm (* Daubney, 1933) 6.5-7.0 mm (*Ransom, 1911 and *Kalantaryan, 1928)	150-180 µm (*Ransom, 1911 and *Kalantaryan, 1928) 220-265 µm (* Daubney, 1933)	sheep (Travassos, 1921, Daubney, 1933, *Kalantaryan, 1928)
	Antilocapridae: Pronghorn	*Ostertagia davtiani*	**Minor** ***davtiani***	Grigoryan, 1951		11.5-12.5 mm (*Grigoryan, 1951) 8.2 mm (Andreeva and Satubaldin 1954)	215 µm (*Grigoryan, 1951) 182-208 µm(Andreeva and Satubaldin 1954)	sheep and goats (Andreeva and Satubaldin 1954) roe deer (Grigoryan 1951)
Trichostrongylus axei	Bovidae: Pronghorn, Bison, Mountain Goat, bighorn sheep, chamois, tahr, cattle, sheep, goat. Cervidae: elk, mule deer, white tail deer, caribou, fallow deer, red deer Camelidae: llama, alpaca Leporidae: rabbit, Equidae: horse, Felidae: cat, Hominidae: human	*Strongylus axei* *Strongylus extenuatus* *Trichostrongylus extenuatus* *Strongylus gracilis*		Cobbold, 1879	4.6-5.5 mm (*Ransom, 1911 and *Kalantaryan, 1928)	3.4-4.5 mm (*Ransom, 1911 and *Kalantaryan, 1928)	smallest=85-104 µm and largest=110-128 µm (*Ransom, 1911 and *Kalantaryan, 1928)	cattle, sheep, goat, bharal, antilope, mule deer, roe deer, caribou (Ransom, 1911)
Trichostrongylus askivali	Red deer, white-tail deer, sambar deer			Dunn, 1964	3-4.2 mm (Dunn 1965)	2.6-3.3 mm (Dunn 1965)	smallest=71-83µm and largest=77-89 µm (Dunn 1965)	red deer (Dunn 1965)

*= in Skryabin (1954)

1.3.3.1.2 *Pathogenicity and clinical signs.*

It is known that *Ostertagia*-type nematodes cause a classical parasitic gastritis (abomasitis) in ruminants resulting in signs of diarrhoea and weight loss (Williams *et al.* 1987). The pathogenicity is well described for sheep and cattle. Observations in deer would indicate the same sequence occurs. Larvae penetrate and then mature in the abomasal gastric glands resulting in distension. The host responds with a focal area of mucosal hyperplasia and cellular metaplasia. After the adult nematodes emerge these cellular changes become more generalized. Together with these changes there is a rapid turnover of cells, loose tight junctions between epithelial cells and increased production of mucus. Collectively these lead to protein-losing gastritis. (Noble 1989; Taylor *et al.* 2007).

The disease can be classified into 3 types in cattle.

Ostertagiosis Type I: In this the larvae complete their development without becoming inhibited within the definitive host. This is the usual presentation of disease caused by these nematodes and is the only type seen in sheep. In deer, the most common is type I.

Ostertagiosis pre-Type II / arrested development. In this the larvae accumulate at the early L4 stage within the mucosal glands. Several factors may stimulate larvae to become inhibited including the exposure of the L3 to adverse environmental situations but it is also necessary that the specific worm has the genetic ability to enter a hypobiotic state, sometimes referred to as diapause (Sommerville and Davey 2002). Larvae usually accumulate over a period of inclement climatic conditions and in New Zealand that is winter. These inhibited larvae do not usually stimulate a host response/clinical signs.

Ostertagiosis Type II: In this the larvae are triggered to synchronously reactivate generating a massive outflow of nematodes from the glands, damaging the mucosa and triggering severe disease. The trigger for the activation and maturation of the larvae is still not very clear, but it may be related to the immunity in the host and obviously in the number of larvae in pre-Type II stage. In deer, Type II disease has only been described in red deer in Britain (Connan 1991) and in the North American Elk in Canada (Woodbury and Parry 2009). To date, it hasn’t been formally reported in New Zealand farmed deer

Ostertagiosis Type II, is not commonly reported in cattle in New Zealand which is most likely due to a combination of factors. Some studies have suggested that inhibition of the L4 stage is genetically explained (Eysker 1997). However, environmental conditions are also considered to be involved (Sommerville and Davey 2002). It is also understood that in New Zealand most inhibited larvae demonstrate a slow reactivation over a longer period (Bisset and Marshall 1987) rather than a synchronous emergence of all inhibited larvae.

Overall, there is only a limited published literature on parasitic disease caused by *Ostertagia*-type nematodes in deer. It is known that *Spiculopteragia* spp. in deer can decrease body condition of infected animals (Kutz *et al.* 2012). In Scotland, deaths were reported in yearling red deer which had an abomasal worm burden dominated by *O. leptospicularis*. (Dunn 1983). *O. leptospicularis* can also infect cattle and sheep (Bisset 1980; Borgsteede 1981; McKenna 2009a) and in the former has high infectivity and pathogenicity (Buel *et al.* 1984), particularly when it is together with *O. ostertagi.* It is also notable that in cattle *O. ostertagi* and *O. leptospicularis* may have different susceptibilities to anthelmintics (Bisset 1980).

Other related nematodes have been reported in red deer including *T. circumcincta* (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b; Manfredi *et al.* 2007; Pato *et al.* 2013) whose preferential hosts are sheep/goats and *Ostertagia ostertagi* (McKenna 2009a) whose preferential hosts are cattle. Although there are reports of clinical disease associated with *O. ostertagi* in white tailed deer in the US (Pursglov *et al.* 1974; Conti and Howerth 1987) none have been reported in red deer to date. Similarly, there are no reports of clinical disease with *T. circumcincta* in red deer.

1.3.3.2 *Other trichostrongyloid nematodes*

1.3.3.2.1 Trichostrongylus spp.

In this genus the nematodes are small. Some species are frequently found in the small intestine and others in the abomasum of ruminants. Table 0.1 compares the features of *T. askivali* and *T. axei* which are the two species commonly found in deer in New Zealand.

Trichostrongylus axei (Cobbold, 1879) has been recorded from a range of grazing species. In at least one comparative study it was found that more L3 established in sheep than cattle, although no statistical evaluation was undertaken (Borgsteede 1981). Until recently in New Zealand, *T. axei* was considered the only member of this genus in the abomasum of red deer. The recognition that *Trichostrongylus askivali* (McKenna 2009c) was also present and that the two are morphologically very similar suggests it is not a recent arrival but has been mistakenly identified up until this time.

The pathogenicity of *T. axei,* as for other trichostrongyloids, is influenced by the number of nematodes present and by the development of the host immune response. Heavy infections will induce plaque-shaped lesions in the abomasum which are due to hyperplasia of the mucosa and in severe cases or long-lasting infections these can develop into shallow ulcers. The clinical signs reported in lambs include diarrhoea, depression in food intake and weight loss. And in severe cases infection can even cause death (Gibson 1954b, 1954a, 1955).

Very small burdens of *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* have been recovered in several studies in red deer in New Zealand (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b) and elsewhere (Pato *et al.* 2013).

1.3.3.2.2 *Haemonchus spp.*

Haemonchus contortus Rudolphi, 1803 have been found in a range of ruminants although their preferred hosts are sheep and goats. Females are 18-32 mm long and males are 13-20mm (Pato 2012). Adults are large compared to other abomasal nematodes. They are haematophagous nematodes and the female's blood-filled intestine is coiled around her white uterus and ovaries leading to this species having the common name of "barber's pole worm". Females have a vulvar flap and males barbed spicules with both having cervical papillae. The spicules are short 370-450 µm in relation with the body length 13-20mm (Pato 2012). They are generally easy to recognise.

Haemonchus placei, **(**Place 1893). Its preferred host is cattle. Morphologically it is similar to *H. contortus* but it has longer spicules 454-470 µm and the females do not have a vulvar flap (Pato 2012). This species has not been described as present in New Zealand.

Haemonchus cause disease mainly in warm tropical and subtropical areas. Larval development requires relatively high temperatures and humidity. Generally outbreaks are positively correlated with rainfall (Taylor *et al.* 2007). New Zealand is towards the end of the climatic range for *H. contortus* and as a consequence *H. contortus* is mainly found in the North Island and then generally only over the warmer summer/autumn months. As for other trichostrongyloids the early L4 of *H. contortus* is also capable of diapause in the abomasum (Sommerville and Davey 2002). The minimum prepatent period in sheep is 18-21 days and 26-28 days in cattle (Wood *et al.* 1995; Taylor *et al.* 2007).

Their pathogenicity is mostly due to their hematophagous feeding behaviour (Holmes 1987) and the consequences are animals in poor condition with anaemia. In New Zealand it is well known in the North Island to be capable of killing sheep and goats. For deer there are only anecdotal comments from clinicians of haemonchosis which have been reported (Swanson *et al.* 2007). Several cases have been reported in white tail deer in the United States (Prestwood and Kellogg 1971; Davidson *et al.* 1980).

1.3.3.2.3 Nematodirus spp.

Nematodirus spp., are found in the small intestine of ruminants. The life cycle of these species differs from other GIN in that the egg is relatively large and the larva develops to the L3 within the egg (Dikmans and Shor 1942). This genus has been reported from deer in New Zealand (Mason 1977) but the species involved were not indicated. Overseas it has been reported to be common (prevalence 66%) in roe deer on the Iberian Peninsula (Pato *et al.* 2013) and to be present in fallow deer in Europe (Rehbein *et al.* 2014). In red deer low numbers of *Nematodirus* spp. have been reported on a few occasions (Irvine *et al.* 2006; Demiaszkiewicz *et al.* 2009). Clinical disease associated with *Nematodirus* infection has not been reported in deer in New Zealand.

1.3.3.2.4 Cooperia spp.

Cooperia spp. are located in the small intestine of ruminants. In temperate zones they rarely cause significant pathogenicity, except when they are associated with other more pathogenic nematodes. Low burdens of different species of *Cooperia* spp. from cattle have been described as present in the small intestine of red deer including *C. oncophora* (McKenna 2009a), *C. punctata* (Connan 1996; Hoskin *et al.* 2000a), *C. pectinata* (McKenna 2009c) and also *C. mcmasteri* (Hoskin *et al.* 2000b). *Cooperia curticei* is another prevalent nematode in sheep and goats but hasn't been reported in natural infections in red deer in New Zealand.

1.3.3.3 Oesophagostomum spp.

Before this PhD thesis, only two species of *Oesophagostomum* were described from the large intestine of red deer in New Zealand. *O. venulosum,* whose preferential host is sheep, is the most prevalent (McKenna 1997; Hoskin *et al.* 2000b; McKenna 2009a). *Oesophagostomum radiatum,* whose preferential host is cattle, has also been reported, but only once in red deer (McKenna 1999). However, *Oesophagostomum sikae* which is morphologically similar to *O. radiatum* has been found in sika deer (Cameron and Parnell 1933; in Popova 1965). It has recently been characterised using molecular tools and identified in red deer and roe deer in the northern hemisphere (Patrelle *et al.* 2014). The first report of this species in New Zealand is made in Chapter 2.

1.3.3.3.1 Description

Oesophagostomum venulosum (Rudolphi, 1809). The male adult length is 11-16 mm and the female 13-24 mm. The head has a buccal capsule with 18 parts in the external leaf crown. This is surrounded by a buccal collar which is separated from the cephalic vesicle by a well-marked groove. It also has a pair of cervical papillae that are located at the end of the oesophagus. The lateral cervical alae are not developed (Taylor *et al.* 2007).

Oesophagostomum radiatum (Rudolphi, 1803). The male adult length is 12-17mm and the female 16-22 mm (Taylor *et al.* 2007). The external leaf crown is absent. An obvious buccal collar is present and is clearly separated from the cephalic vesicle. The cephalic vesicle has a small constriction at the beginning of the distal third. Lateral alae extend down below the cephalic vesicle. The cervical papillae are located in the middle of the oesophagus. The gubernaculum is 0.1 mm long (cited by Goodey 1924, in Popova 1965).

Oesophagostomum sikae (Cameron and Parnell, 1933). The adult male length is 9.95-12mm and the female 12.9-17.25mm long (Yamaguti 1935; Popova 1965). The external leaf crown is absent. An obvious buccal collar is present. The cephalic vesicle is similar to *O. radiatum* but is constricted in the middle (Yamaguti 1935). The cephalic portion of the nematode is curved ventrally. According to Popova (1965) the key morphological difference with *O. radiatum* is that *O. sikae* doesn't have a gubernaculum and in the oesophageal funnel it has three lanceolate formations that are absent in the oesophagus of *O. radiatum*.

1.3.3.3.2 Epidemiology

The epidemiology of *O. venulosum* in sheep and *O. radiatum* in cattle is similar to the trichostrongylids where the L4 is able to enter diapause in the mucosa. In its free-living stage the L3 is also able to survive on pasture over winter in temperate areas (Taylor *et al.* 2007). The minimum prepatent period for both species is around 35-42 days (Wood *et al.* 1995). *O. venulosum* is considered to prefer temperate climates whereas *O. radiatum* is more common in tropical and subtropical areas. Cattle are considered to be able to develop good immunity after exposure (Taylor *et al.* 2007) whereas available information indicates that sheep are able to develop a good immune response against developing worms but not against adults (Dash 1981).

1.3.3.3.3 Pathogenicity and clinical signs

O. venulosum in sheep is not very pathogenic and is not known to induce nodule formation around the larvae within the mucosa in naturally infected sheep. However, nodule formation has been reported in at least two studies with experimentally infected sheep (Goldberg 1952; Clark *et al.* 1978). In comparison, cattle do develop a pronounced inflammatory nodule around *O. radiatum* larvae within the mucosa. A large number of nodules can cause considerable damage (Taylor *et al.* 2007). There are no reports on the pathogenicity of any *Oesophagostomum* spp. in deer.

1.3.3.4 Chabertia sp.

Chabertia ovina infects the large intestine of sheep and goats. It is only occasionally reported from deer or cattle with resulting problems ascribed to this species. In red deer in New Zealand it has only been described as present in low burdens in artificially infected deer (Mackintosh *et al.* 1997). In a study in Poland, it was described as highly prevalent in cervids including red deer (Burlinski *et al.* 2011). However, the authors undertook only morphological identification of the infective larvae from cultured eggs and as *Chabertia* L3 are known to be very similar to those from the *Oesophagostomum* genus, and since some authors have stated that it is impossible to differentiate between them (Patrelle *et al.* 2014) these may have been misidentified. This species is commonly called the "large-mouthed bowel worm", as it has a large buccal capsule which it uses for attachment and feeding on the tissue of the colonic mucosa. Because of the damage in the mucosa resulting in local haemorrhage and also protein losses, this worm can be very pathogenic, especially when burdens are around 300 (Taylor *et al.* 2007).

1.4 Diagnostic Techniques for GIN

A good diagnostic technique is essential for good control of parasites, as clinical signs of helminth disease are not very specific. Morphological methods for the identification of adult stages can only be used at post mortem. In live animals the options are limited to quantifying eggs in faeces with the possibility of subsequent coproculture to identify any developed larvae to the genus level. More recently molecular methods for the identification of adults and immature stages (both in and outside the animal) have been developed and are now being used when precise identification is required. The same ranges of techniques are used for deer as for other ruminants.

1.4.1 Morphological diagnostics

1.4.1.1 Coprological

Examination of faecal samples is the most commonly used method to estimate the worm burden because it is cheap, fast and easy to perform. There are several methods available, including flotation methods (direct flotation, McMaster methods, Flotac), sedimentation methods (Baermann technique), and many modifications of them.

These methods have been used to measure: the status of helminth infection in the herd (Ward *et al.* 1997); the presence of anthelmintic resistance (Coles *et al.* 1992); the subsequent level of pasture contamination; and different aspects of the epidemiology of helminth infection (Vadlejch *et al.* 2011). Even though it is of great investigative value, it has some limitations. The rate of egg or larva shedding may vary depending on several factors including the age of the animal, faecal moisture content and development of immunity due to past exposure. As most strongylid species produce similar eggs, morphological identification is not possible to species level and counting eggs only allows a quantitative diagnosis at a superficial level. If coproculture is undertaken, the resulting larvae can be identified morphologically to genus level. Final diagnosis must take into account other factors including the clinical signs or pathological impact on the animal and host species (Gasser 2006).

1.4.1.1.1 Faecal egg count (FEC).

This diagnostic method is the most commonly used, because it is easy to perform and relatively cheap using only basic laboratory instruments. To execute a FEC, it is necessary to mix a measured quantity of faeces with a flotation solution and an aliquot of this solution is examined under a microscope in a counting chamber. To estimate the number of eggs per gram (eggs/g) of faeces, the McMaster technique is by far the most popular (Roeber *et al.* 2013b).

1.4.1.1.2 McMaster technique

It is a quantification diagnostic method, originally described by Gordon and Whitlock (1939) and it has been modified many times (Table 0.2). Most variations use a slide with two compartments with grids under the top slide. The compartments are filled with a known faecal content in a flotation solution, and the eggs that float up within the grid under the top slide are counted. Variations include different flotation solutions with different specific gravities, the amount of faeces used, application or not of centrifugation, the number of counting chambers, and the resulting dilution factors (Vadlejch *et al.* 2011).

Table 0.2 A selection of variations of Modifications of the McMaster technique by different authors.

	Hodges *et al.* (1983, in Stafford *et al.*	Wetzel (1951)	Zajíček (1978)	Roepstorff and Nansen (1998)

	1994)			
Faeces (g)	2	2	1	4
Centrifugation	No	No	yes	Yes
Flotation Solution (ml)	28 ml NaCl	60 ml NaCl	10 ml $MgSO_4$+$Na_2S_2O_3$	4 ml NaCl+Glucose
Solution specific gravity	1.2	1.2	1.28	1.3
Flotation rest time	3 min	2-3 min	5 min	3-5 min
Chambers counted	2	3	2	2
Egg multiplied by coefficient	50	67	33	20
Dilution factor	1:14	1:30	1: 5	1:14

Changes in the technique vary the sensitivity of the method. When larger amounts of faeces are used, the technique becomes more sensitive. Some authors described an optimal dilution factor of around 1:10-1:15 (Vadlejch *et al.* 2011) explaining that when the dilution factor is too high, the eggs are not always detected and when it is too low, the probability of overlooking eggs by the technician increases (Vadlejch *et al.* 2011). When centrifugation is included, the method becomes more reliable but is more complex and labour intensive. A comparison of the three modified methods (by Wetzel (1951), by Zajíček (1978) and by Roepstorff and Nansen (1998)) are shown in Table 0.2. When samples were ≥ 200 eggs/g the three methods were reliable with 100% sensitivity. However, when ≤ 20 eggs/g, none of them were able to detect 100% of the positive samples. The method by Roepstorff and Nansen (1998) was the most sensitive (70%).

The relationship between egg counts and worm burdens in deer is considered to be poor (Mackintosh and Tolentino 2009). Nevertheless, in a study on anthelmintic efficacy by Mackintosh *et al.* (2014b) it was found that there was a high correlation between *Ostertagia*-type egg output and worm burden in young deer. However, in the same study this correlation was low after treatment with moxidectin. To explain the low correlation in treated deer, the authors conclude that a temporary suppression of the production of eggs in the resistant nematodes may have occured (Mackintosh *et al.* 2014b).

1.4.1.1.3 Faecal larvae count (FLC).

For the estimation of the lungworm larvae from faeces, sedimentation methods such as the Baermann technique are frequently used. The Baermann technique usually consists of a funnel with a clipped tube attached at the bottom end or a conical flask with a rounded bottom. A known amount of faecal sample is inserted over a mesh/gauze/filter paper at the top. The funnel/conical flask is filled with water to cover the faecal material. This is incubated for a variable period between "overnight" to 20 hrs at "room temperature". The active larvae will pass through the mesh/gauze/filter paper and collect at the bottom of the funnel/conical flask. Depending on the apparatus used the larvae are either collected by opening the bottom of the clipped tube or by siphoning the fluid from the top of the flask. Larvae are subsequently counted and number per gram calculated. In deer the FLC for lungworm has a good correlation with the actual worm burden, making it a reliable diagnostic technique (Mackintosh *et al.* 2014b).

1.4.1.1.4 Culture and larval speciation

The eggs of GIN require aeration and an appropriate warm temperature and humidity to develop into infective L3. To provide the resources for the development of the larvae a variety of techniques have been described. These involve a culture for 7-14 days at temperatures ranging from 21-28ºC. Faeces are initially mixed with an inert material to facilitate aeration which could be sawdust, dried sphagnum moss, sterile cattle, sheep or horse faeces (Roberts and O'Sullivan 1950) and vermiculite. The latter is the most popular because it is cheap, easy to obtain and its porous nature facilitates distribution of air and water through the mixture. (Porter *et al.* 1965). After culture several different techniques have been described to recover the larvae. Most use some variation of the Baermann Technique (Berrie *et al.* 1988; Taylor *et al.* 2007). Larvae are then identified according to readily available keys (MAFF 1977) which describe the features of species found in sheep and cattle. However, the characteristics of L3 of *Ostertagia*-type species from deer haven't been described in the literature.

1.4.1.2 Nematode burdens

If animals are killed it is possible to undertake a count of the actual nematode burden. This generally involves washing the organs separately and counting the worms in an aliquot. The most commonly used approach is that described in the World Association for the Advancement of Veterinary Parasitology guidelines for evaluating the efficacy of anthelmintics (Wood *et al.* 1995). This involves separately washing each organ of the gastrointestinal tract and counting the worms in a 5% aliquot. This aliquot is sieved through a 38 µm aperture sieve to retain all larval stages. If many worms are expected the size of the aliquot can be decreased (Clark *et al.* 1971) and inversely if only a few worms are present the size of the aliquot can be increased (Wood *et al.* 1995). To recover the immature worms, especially *Ostertagia*-type species, from deep within the mucosa two different approaches have been described.

1. The incubation method. This can only be used with a fresh abomasum. The abomasal mucosa is soaked face down in saline for 24 hrs or overnight under warm conditions (usually 37-40ºC) and the larvae that emerge are recovered from the water.
2. The digestion method. For this the mucosa is immersed in a solution of 1% pepsin in 3% hydrochloric acid (HCl) or just 3% HCl. This is then incubated for 4-6 hrs at 37-40ºC. The digested mucosal material is later sieved (38 µm) and a 5% aliquot is counted under the dissecting microscope. Wood *et al.* (1995) confirmed that 10% more larvae were recovered with the digestion method than the incubation method.

For the recovery of *Dictyocaulus* spp. three general techniques have been described.

1. Perfusion method. This method was originally described by Inderbitzin (1976; cited by Oakley 1980) and modified by Hoskin *et al.* (2000b). For this the lungs must be intact and still together with the pulmonary artery and at least part of the trachea. A hose is placed within the pulmonary artery and securely tied in place.

At least 30 litres of cold water is then run through the lungs at a constant pressure of 2.11 kg/cm^2. This is generally equivalent to normal household water pressure. The recovered liquid is passed through a 53 µm sieve to recover the nematodes.

2. Dissection, washing. For this technique the lungs are dissected and individual pieces are washed with water over a fine sieve.
3. Modified Baermann technique. Similar to the dissection technique, lungs are dissected and washed in normal saline soon after death. The lungs are later immersed in normal saline at 40°C for an hour and then cut pieces of lung are inserted in a Baermann apparatus. The resulting normal saline collected is then sieved and the lungworms counted.

Oakley (1980) compared a modified technique of the perfusion method, with a modified Baermann technique and proved that the Inderbitzin method was more efficient for the recovery of *D. viviparus.*

1.4.2 Molecular methods

Molecular biological methods are now being used in parasitology, although their use for routine diagnostic purposes is still very limited. They provide the opportunity to unambiguously identify parasites of any sex and stage if the appropriate information about their DNA is available. The most commonly used technique involves the polymerase chain reaction (PCR).

1.4.2.1 PCR

This technique was developed and described by Mullis *et al.* (1986) and later improved by Saiki *et al* (1988) with the use of the thermostable DNA polymerase enzyme isolated from *Thermus aquaticus* (taq). To amplify a piece of nucleic acid this technique relies on thermal cycling. Initially the DNA is heated to denature the double stranded genomic DNA. This step is followed by a temperature decrease to allow for the annealing or hybridization of the primers to the DNA strands. The next step follows a rise in temperature to promote extension of the DNA from the primers using a polymerase, usually taq (Gasser 2006). A number of variations of this technique have been described. More recently, real time PCR (qPCR) is the technique that has been reported for use with identifying parasites. This technique is able to quantify the amount of parasite DNA present.

1.4.2.1.1 PCR in Nematodes

A suitable DNA target region is required to be identified that is stable within a species but has enough variability at the level needed to identify that species. The most common targets that have been used are the first internal transcribed spacer (ITS-1) and the second internal transcribed spacer (ITS-2) of the nuclear ribosomal DNA region (rDNA) (Bott *et al.* 2009; Roeber *et al.* 2012a; Roeber *et al.* 2013a; Bisset *et al.* 2014). These regions are known to have enough variability at species level together with low intraspecific variation.

In sheep, for the identification of eggs, Bott *et al.* (2009), developed a semi-quantitative qPCR protocol, where ITS-2 was used as the target region. The eggs were separated by flotation from the faecal sample followed by extraction of the DNA by column-purification. For amplification two primers were used, a forward species-specific primer in the ITS-2 region and a conserved reverse primer in the 5' region of the large subunit of rDNA. The authors found a correlation between the eggs per gram in the FEC and the values in the qPCR. These authors claimed this method was more sensitive than the McMaster method.

In sheep, Bisset *et al.* (2014) developed a multiplex PCR method to identify the species of nematodes commonly found in sheep in New Zealand. They recovered DNA from individual L3 and used the ITS-2 region as the target region. For this method they included 5 species in each multiplex reaction and thus required two reactions to cover all sheep nematode species of interest. Each multiplex reaction included either reverse or forward primers for each species, together with two conserved "generic" primers (reverse and forward). Consequently, two products were amplified, one for the "generic" and another for the species-specific primer. The former confirms that strongylid DNA is present and the latter if one of the five species is present. The range of species described included sheep nematode species and also *O. ostertagi, C. oncophora, C. punctata* from cattle and *O. leptospicularis* from deer.

The same approach was taken with some deer species but this was only described in overview (Knight *et al.* 2011). They were not considered with other sheep and cattle species to indicate whether the primer sets would exclude sheep and/or cattle nematode species.

1.5 Control

1.5.1 Anthelmintics:

The development of the first broad spectrum anthelmintic thiabendazole (benzimidazole) in 1961 changed the way to treat parasites. Since then, numerous drugs have been developed (Lanusse and Prichard 1993). Anthelmintics can be classified by their chemical structure and/or their mode of action.

In New Zealand there are only two action families of anthelmintics registered for deer.

- Benzimidazoles (BZ): albendazole, fenbendazole, and oxfendazole are currently licenced for use in deer using the same dose rates as for cattle except for albendazole for which the dose rate is 33% higher than for cattle.
- Macrocyclic lactones (ML): ivermectin, abamectin, eprinomectin and moxidectin are currently licenced for use in deer but only as topical pour-on formulations using the same dose rates as for cattle. There is little information on the efficacy of other formulations.

Another broad spectrum action family includes levamisole. In the early 1980s a pilot trial on deer infected with *Dictyocaulus* found that levamisole had a reduced efficacy compared with cattle, suggesting that deer metabolized this drug faster (Mason 1982). In a residue study where deer (n=9) were treated with a triple combination including injectable moxidectin (2mg/kg), oral oxfendazole (18.12mg/kg) plus oral levamisole (15mg/ kg) one animal had high levels of levamisole in fat 42 days post treatment, even though the withholding time of levamisole in deer was speculated to be short (Lawrence 2015). This study illustrates the lack of understanding of anthelmintics in deer, especially in combination. The study authors recommended not using these products together within 42 days of slaughter.

The issue of speed of metabolism has also been explored for the other action families. Watson and Manley (1985) observed that deer metabolized oxfendazole faster than sheep. Similarly, Mackintosh *et al.* (1985) in a study on the pharmacokinetics and efficacy of ivermectin and febantel observed that deer metabolised febantel faster than cattle and sheep but the pharmacokinetic parameters for ivermectin were similar to those in cattle. This would appear to be the only published information on pharmacokinetics of MLs in deer.

For efficient parasite control there should be a solid understanding of the pharmacokinetics of the anthelmintic drugs to ensure an effective dose is used together with an understanding of local nematode epidemiology so that factors such as weather, season, stocking rate, age of the host, farm type can be considered. Relying only on anthelmintics has led to an increase in the prevalence of anthelmintic resistance around the world in all grazing ruminants. Deer farms in New Zealand are no exception. Indeed, there is recent evidence of widespread resistance in the *Ostertagia*-type nematodes to ML anthelmintics (Lawrence 2011; Lawrence *et al.* 2012; Hodgson 2013; Lawrence *et al.* 2013; Mackintosh *et al.* 2013) and there is a report where there is clear evidence of resistance to oxfendazole (Lawrence *et al.* 2013). This has posed significant challenges to deer farmers, given that there are no other anthelmintics registered for deer in New Zealand. Therefore, there is a growing interest to develop alternative control methods which rely less on anthelmintic drugs.

1.5.2 Alternative methods

Some of the alternative methods to control nematodes are explained below.

1.5.2.1 Manipulating host immunity

1.5.2.1.1 Vaccination

An attenuated vaccine against Dictyocaulus viviparus in cattle (Jarrett and Sharp 1963) has not proven to be fully protective against lungworm in deer (Johnson et al. 2003a). The recent development of a commercial subunit vaccine against *Haemonchus contortus* in sheep "Barbervax®" would not be useful for deer as this species is a minor parasite in this host.

1.5.2.1.2 Improving the nutritional status of the animals

During periods when the animal has high demands for nutrients, e.g. the periparturient period and also young animals growing rapidly, the immunological response has a lower priority for available nutrients (Coop and Kyriazakis 1999). If the animals are supplemented during these periods there can be a beneficial effect on parasite resistance and/or resilience by the host. Protein supplementation has been shown to decrease FEC and worm burdens in merino ewes (Kahn *et al.* 2003). However in the same study, the effect of supplementation for animals with a resistant genotype was less effective than for randomly selected animals (Kahn *et al.* 2003).

1.5.2.1.3 *Resistant genotypes*

There are several examples where sheep have been selected for enhanced resistance to nematodes. AgResearch developed lines of Romney sheep selected for high and low faecal egg counts (Bisset *et al.* 1994; Bisset *et al.* 1996; Bisset *et al.* 2001). Similar, lines of Merino sheep selected for enhanced resistance to *Haemonchus contortus* have been selected in Australia (Woolaston 1992; Kahn *et al.* 2003).

1.5.2.2 Forages with nutraceutical properties

Some plants with higher content of condensed tannins (CT) have been considered to have anthelmintic properties including *Acacia karoo* "Sweet Thorn" (Kahiya *et al.* 2003), *Lysiloma latisiliquum* "False Tamarind" (Brunet *et al.* 2008), *Lespedeza cuneata* "Chinese Bushclover" (Shaik *et al.* 2006; Terrill *et al.* 2009), *Acacia molissima* "Sydney Wattle" (Minho *et al.* 2008), *Manihot esculenta* "Cassava", *Hedysarum coronarium* "Sulla", *Onobrychis viciifoliae* "Sainfoin", *Lotus pedunculatus* "Big Trefoil", *Lotus corniculatus* "Birdfoot Trefoil", *Cichorium intybus* "Chicory" (Hoste *et al.* 2006), *Salix* spp. "Willow" (Mupeyo *et al.* 2011). The evidence for the anthelmintic properties of these plants is variable and some are only from *in vitro* studies. Some of this variation will be due to each plant having their own chemical characteristics, concentration of CT and different mode of action (Naumann *et al.* 2013). There may also be a difference in the response of each species of nematode (Hoste *et al.* 2006). There is some evidence that condensed tannins may bind eggs in faecal material without killing them but making them less likely to float in a faecal egg count (Mupeyo *et al.* 2011). Sesquiterpenes, as found in Chicory, are another group of plant secondary metabolites which have been considered to have anthelmintic properties for deer nematodes *in vitro* (Molan *et al.* 2003).

In a study of deer artificially infected with lungworm and GIN which were fed with three different forage legumes (lucerne, birdsfoot trefoil and sulla), the group fed sulla that contained the highest percentage of condensed tannins, had fewer abomasal nematodes ($p<0.05$) than the other groups but the lungworm burdens were not different ($p>0.05$) between the groups (Hoskin *et al.* 2000a).

1.5.2.3 Biological control:

The use of biological control agents such as *Duddingtonia flagrans*, a micro-fungus able to trap larvae in faeces, has been investigated in many different experiments in recent years. For example, experiments in sheep have demonstrated that *D. flagrans* spores are able to survive passage through the gastrointestinal tract (Larsen et al. 1998) and are then subsequently able to reduce the number of infective larvae in faeces in the environment (Faedo et al. 1997; Faedo et al. 1998; Waller et al. 2001). To date there has been no successful commercial product where a mechanism allowing slow release of spores has been developed for grazing ruminants.

1.5.2.4 Grazing management:

Grazing management has been investigated many times for control of GIN. Hoste and Torres-Acosta (2011) proposed two main types of grazing strategies which they classified as defensive or offensive.

- Defensive: To avoid parasites
  - Decrease the stocking rate (diluting) or rotational grazing (evasive).
- Offensive. To decrease the availability of nematodes by removal of infective stages on pasture.
  - Grazing alternate species which are not hosts for the target nematodes (Hoste and Torres-Acosta 2011).

Cross-grazing

The principle that underlies this approach is that most nematode species have a preferential host and if they are ingested by another host, they die or they establish at a lower rate, consequently restricting the number of L3 on pasture. The value of cross/co-grazing by different species for pasture decontamination has been described by several authors – see Appendix 1 Section 1 for a summary of field studies on this aspect. Reviewing all these studies it is apparent that the main aim of these field studies was decontamination of sheep pasture by grazing with cattle (Arundel and Hamilton 1975; Donald *et al.* 1987) or cattle pasture by grazing with sheep (Barger and Southcott 1975) or both (Southcott and Barger 1975; Helle 1981; Inderbitzin *et al.* 1981; Jordan *et al.* 1988). The design of the studies depended on their specific objectives, some of them focusing on how often to alternate (Barger and Southcott 1975; Southcott and Barger 1975; Barger and Southcott 1978), others on the different proportion and stocking rate of the two ruminants species to achieve effective nematode control (Arundel and Hamilton 1975) whilst another investigated the balance between the quality of pasture composition versus the reduction of nematodes (Moss *et al.* 1998). Only one field study has been reported in New Zealand investigating this option (Moss *et al.* 1998).

Only some of these studies were replicated (Barger and Southcott 1978; Jordan *et al.* 1988; Marley *et al.* 2006; Bailey *et al.* 2009; Mahieu 2013) which limits the ability to statistically analyse those without replication. Also, most of the studies were executed without rotation of paddocks, particularly the earlier studies (Arundel and Hamilton 1975; Barger and Southcott 1975; Southcott and Barger 1975; Barger and Southcott 1978; Helle 1981; Donald *et al.* 1987; Jordan *et al.* 1988; Bairden *et al.* 1995; Amarante *et al.* 1997; Marley *et al.* 2006). More recently many do include rotation (Inderbitzin *et al.* 1981; Moss *et al.* 1998; Rocha *et al.* 2008; Bailey *et al.* 2009; Mahieu and Aumont 2009; Sormunen-Cristian *et al.* 2012; Mahieu 2013) but it does complicate the design and execution of such studies.

With a few exceptions, most of the studies found that cross infection of nematodes between cattle and sheep/goats was low and vice versa. In Australian studies, the pathogenic nematode *O. ostertagi* was reduced following cross grazing with sheep (Barger and Southcott 1975; Southcott and Barger 1975) and as this is considered to be the most pathogenic species in cattle, it was indicating a positive effect. Reductions were also noted in *C. oncophora* burdens (Southcott and Barger 1975) but as these are considered to be less pathogenic, they are of less importance. For sheep, cross-grazing has demonstrated a reduction in three of the more pathogenic species being *T. circumcincta* (Arundel and Hamilton 1975; Southcott and Barger 1975; Donald *et al.* 1987), *H. contortus* (Southcott and Barger 1975; Barger and Southcott 1978; Donald *et al.* 1987; Amarante *et al.* 1997; Mahieu and Aumont 2009) and *T. colubriformis* (Southcott and Barger 1975; Barger and Southcott 1978; Amarante *et al.* 1997). None has demonstrated a significant effect on the small intestinal nematode *T. vitrinus,* but that is largely because in the studies this species was not present or it was in low numbers. The abomasal worm *T. axei* is capable of infecting a variety of species including sheep, cattle and deer. Worm burdens in cattle when co/cross-grazing with sheep resulted in a reduction of *T. axei*, but in sheep co/cross-grazing with cattle the results are somewhat contradictory. In two studies in Australia one showed reductions of *T. axei* in sheep when cross-grazing with cattle (Barger and Southcott 1978) whilst a second found higher burdens when co-grazing with cattle (Arundel and Hamilton 1975). The differences observed in these studies may be related to systems used, cross-grazing versus co-grazing. There is also a suggestion that some strains of *T. axei* have a predilection for sheep or cattle which may have influenced these results

In some studies the prevalence of *C. oncophora* in sheep was greater when they were grazing with cattle than on their own (Arundel and Hamilton 1975; Barger and Southcott 1978; Mahieu and Aumont 2009). Similarly, higher numbers of *H. contortus* were reported in cattle when co/cross-grazing sheep (Southcott and Barger 1975; Mahieu and Aumont 2009). For both these species the alternate ruminant is considered a secondary host so the finding is not unexpected but does illustrate that such co/cross-grazing systems can result in negative outcomes for the grazing animals.

When comparing egg counts for mono-grazing, cross-grazing or co-grazing, some studies found differences only in some months, others over the whole study and others no differences. In the UK, Marley *et al.* (2006) found that for lambs cross-grazing with cattle, the egg counts were lower compared to lambs co-grazing with cattle and mono-grazing on their own. In Australia, Bailey *et al.* (2009) and Barger and Southcott (1978) found lower egg counts in lambs cross-grazing with cattle compared with the control lambs. Similarly, Mahieu and Aumont (2009) in the Caribbean observed that young lambs cross-grazing with cattle had lower egg counts than mono-grazing lambs during the dry and rainy season and the same differences were seen in older lambs during all seasons, except in weaners in the rainy season. Egg counts in calves that were cross-grazing with sheep in Norway were lower compared with those mono-grazing (Helle 1981). Similarly, in a study in Switzerland, differences between mono- and cross-grazing in calves were seen only in some months in the third and fourth year of the study and mono-grazing had higher counts than cross-grazing (Inderbitzin *et al.* 1981). Overall, there is generally some benefit in terms of egg counts seen at some times in all these studies but the results do vary.

Regardless of egg counts the real interest is in differences in worm burdens or more importantly weight gains. Interestingly, even if egg counts in sheep co-grazing with cattle were higher than when they were cross-grazing, the liveweight of the lambs behaved inversely and lambs co-grazing had higher weights compared with cross-grazing (Helle 1981; Marley *et al.* 2006) or mono-grazing lambs (Jordan *et al.* 1988), indicating that there is also a pasture quality effect when the two species are sharing grassland. Liveweight of calves were superior when cross-grazing compared to co-grazing with sheep (Helle 1981) and Jordan *et al.* (1988) found that even if the calves in the co-grazing group had lower egg counts than the controls, they still had lower liveweight. However, not all studies found positive effects on egg counts or worm burdens. In New Zealand Moss *et al.* (1998) did not find any significance difference in egg counts or in worm burdens, but they did find a significant effect in the reduction of pasture larval numbers when cattle grazed sheep pasture and also an increase in lamb carcass weight. They concluded that the increase in lamb weight was largely due to the improved pasture quality following cattle grazing.

One study in the UK reported reductions in worm burdens in the initial 2 years of the study but not in the third or fourth year (Bairden *et al.* 1995), which they indicated was most likely due to larvae surviving on pasture in sufficient numbers over the 18 month period until the pasture was grazed by the same host species again. It is not stated if there was a growth rate advantage in the first two years concurrent with the reduced worm burdens.

As discussed above a review of the usefulness of co/cross-grazing systems endeavours to merge a number of different, often confounding factors together to achieve a useful result. There are differences in pathogenicity between nematode species, the various studies usually concentrate on the more pathogenic ones. Environmental conditions play an important role in the epidemiology in different seasons and climates, *Haemonchus* and *Cooperia* are more prevalent in subtropical and tropical climates, *Ostertagia* and *Nematodirus* in temperate regions and *Trichostrongylus* can be found throughout a variety of climates. Apart from the co/cross-grazing system, other grazing management factors can also influence the mixture and the quantity of nematodes. Higher stocking rates will increase the infection rate and more extensive management will decrease the infectivity; better nutritional status of the animals will increase the immunological response and/or resilience of the animals. Another factor that can modify the results is the age of the animals and their previous exposure to nematodes. Therefore, to have an effective parasite control with co/cross-grazing management and to be able to compare the different approaches in these studies, all of these factors must take into account.

Even though there are many studies on cross-grazing or co-grazing between sheep and cattle, there are no studies on the usefulness of cross-grazing between deer and other ruminant animals as a means of parasite control for deer.

1.6 Summary

In summary, this literature review includes information pertinent to the investigations of this thesis which were necessary to implement and to understand the results of the different studies. Firstly, it provides a general introduction and history of deer farming. Later it describes in detail published material on some aspects of deer nematodes including pathogenicity, diagnosis and control with emphasis on gastrointestinal nematodes. It describes a range of nematodes species that are found in deer including nematodes of cattle and sheep, giving related information about pathogenicity, epidemiology and their life cycle. Additionally, it provides information about different diagnostic techniques for assessing both qualitative and quantitative identification of nematodes. Finally, this literature review covers some aspects of nematode control with emphasis on cross-grazing including a wide range of important information on nematodes on farmed deer.

Chapter 2 Analysis of the pathogenicity and diagnosis of GI nematode infections in young farmed red deer – Initial Study

2.1 Introduction

The impact of gastrointestinal nematodes (GIN) on young deer is poorly understood. It is known that deer are infected by a relatively limited range of species which are mainly concentrated in the abomasum, including deer-specific nematodes in the sub-family Ostertaginae (=*Ostertagia*-type; *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera* and *Ostertagia leptospicularis*) and *Trichostrongylus* spp. Also, deer can be infected with some GIN of sheep and cattle including; *Haemonchus contortus, Oesophagostomum venulosum, Teladorsagia circumcincta, Trichostrongylus vitrinus, Nematodirus* spp.*, Chabertia ovina, Cooperia oncophora. Cooperia punctata*, *Cooperia pectinata* and *Cooperia curticei* (McKenna 2009a; Tapia-Escárate *et al.* 2015b; Chapter 5).

Since the commencement of deer farming in New Zealand in the late 1960s it has been recognised that lungworm was an important helminth pathogen. In these early days of deer farming the impact of gastrointestinal parasitism appeared minimal and they would be controlled in young deer as they were treated for *Dictyocaulus*. However, the impact of gastrointestinal parasites has likely been underestimated and recent evidence of widespread resistance by the *Ostertagia*-type nematodes to ML anthelmintics (Lawrence 2011; Lawrence *et al.* 2012; Hodgson 2013; Lawrence *et al.* 2013; Mackintosh *et al.* 2013) and evidence of resistance to oxfendazole in these same nematodes (Lawrence *et al.* 2013) has increased the relative importance of this group of nematodes. Early studies in deer in New Zealand did not separate GIN as they only evaluated the combined impact of both lungworm together with GIN (Wagner and Mackintosh 1993b; Waldrup and Mackintosh 1993; Waldrup *et al.* 1994; Hoskin *et al.* 2000a; Hoskin *et al.* 2000b) and no studies have been reported involving just infections with gastrointestinal nematodes.

The aim of this study was to determine the relative importance of different levels of artificial infection with a mixed challenge of GIN in young housed deer and to obtain additional information about the relationship between clinical pathology and worm burdens.

2.2 Materials and methods

2.2.1 Experimental Design

The overall study design involved 45 deer allocated into three treatment groups (n=11 each) and one control group (n=12). Deer in the treatment groups were trickle infected 3 times per week with a mixed culture of GIN. The treatment groups comprised one group given a High Dose (HD) challenge, the second a Medium Dose (MD) challenge and the third a Low Dose (LD) challenge. Infections were originally intended to proceed for seven weeks with a further four weeks to allow parasites to mature. However, development of clinical signs shortened the time over which the trickle infection continued. The occurrence of clinical disease also prompted a supplementary experiment utilising the original control group of animals which is described in Chapter 3.

The schedule of events for the trial is shown in Table 0.1. Several deer in the HD and MD groups displayed clinical signs of parasitism and were slaughtered for worm counts, 21 and 18 days, respectively, earlier than planned**.**

Table 0.1 Schedule of treatment, management events and measurements.

Days of Trial	Event
-35 - 0	45 deer weighed, and allocated to 8 pens of 5–6 deer each to adjust to group housing and lucerne chaff-based diet. Weekly: sampling for faecal egg and larvae counts, group mean voluntary food intake (VFI) and individual liveweight gain (LWG).
1-7	Groups randomly assigned to treatment. VFI and liveweight measured, faecal and blood samples obtained, and group VFI. Trickle infection 3x per week.

Days of Trial	Event
8-14	Trickle infection 3x per week. Weekly measurement of weights, faecal and blood samples and group VFI.
15-21	Trickle infection 3x per week. Weekly measurement of weights, faecal and blood samples and group VFI.
22-28	Trickle infection 3x per week. Weekly measurement of weights, faecal and blood samples and group VFI. HD group euthanased for worm counts on Day 28.
29-35	Trickle infection 3x per week, MD group euthanased for worm counts on Day 31
36-38	Low treatment group euthanased for worm counts on Day 38

2.2.2 Animals

Weaner red deer (*Cervus elaphus,* n=45) were purchased from several sources. All animals had been reared on pasture up to the start of the study. On arrival all were effectively treated with an anthelmintic, ranked by weight and randomly allocated to groups with 5-6 animals per indoor pen on sawdust bedding. Animals in each pen were on the same treatment group. During the study animals were fed a combination of commercially available baleage (FibreEzy®, Fibre Fresh Feeds Ltd., Reporoa, New Zealand) supplemented with oats.

2.2.3 Source of Larvae and treatments

Larvae were obtained from six Massey University-born weaner red deer naturally infected fitted with faecal collection bags. To increase the faecal egg counts and minimize the time needed for sample collection these donor deer were given intramuscular dexamethasone sodium phosphate (0.25mg/kg twice a week). The collected faeces were mixed with vermiculite and cultured at 23°C for 2 weeks. The infective larvae were recovered with a standard Baermann apparatus. The same source of larvae was used for all the treatment groups. Three groups of larvae (100 each) were identified morphologically and comprised 0.66% (range=0-1%) *Haemonchus*, 6%

(range=3-8%) *Trichostrongylus*, 40% (range=38-42%) *Ostertagia*-type and 53% (range=52-54%) *Oesophagostomum*. The number of larvae given to each group was calculated for the *Ostertagia*-type component and are shown in Table 0.2 . The dose rates for *Ostertagia*-type nematodes used in this experiment for the HD Group were similar to those used by Hoskin *et al*. (2000b) where no clinical signs were observed, but an effect on growth rate was seen.

Table 0.2 Treatment regime showing number of larvae per individual dose as given three times per week and the total number of larvae given during the experimental period.

Treatment Group	Dose of *Ostertagia*-type L3		Dose of *Oesophagostomum* spp. L3		Dose of *Haemonchus* sp. L3		Dose of *Trichostrongylus* spp. L3	
	Individual (range)	Total (range)	Individual (range)	Total (range)	Individual (range)	Total (range)	Individual (range)	Total (range)
HD	2500 (2375-2625)	30000 (28500-31500)	3333 (3250-3375)	40000 (3900-40500)	42 (0-63)	500 (0-750)	375 (188-500)	4500 (2250-6000)
MD	1500 (1425-1575)	19500 (18525-20475)	2000 (1950-2025)	26000 (25350-26325)	25 (0-38)	325 (0-488)	225 (113-300)	2925 (1463-3900)
LD	500 (475-525)	8000 (7600-8400)	667 (650-675)	10667 (10400-10800)	8 (0-13)	133 (0-200)	75 (38-100)	1200 (600-1600)

2.2.4 Measurements

Liveweight was measured weekly for liveweight gain, and voluntary feed intake (VFI=Offered-Rejected) was measured by pen (5-6 animals/pen), on a daily basis. To crudely estimate individual voluntary feed intake, the feed consumed per pen was divided by the number animals in that pen. Blood samples were collected into plain (until Day 29) and EDTA (until Day 22) vacutainers via jugular venepuncture while deer were physically restrained in a pen. Blood parameters including total serum protein, serum albumin and globulin, white blood cell counts (including individual components; neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cells, haemoglobin and packed cell volume were calculated in a commercial laboratory (New Zealand Veterinary Pathology) using equipment calibrated for deer samples.

Pepsinogen concentrations were determined using a method that has been validated previously in sheep (Scott *et al.* 1995) after adopting minor modifications (Simcock *et al.* 1999). The method is based on the digestion of glycine-buffered BSA following the conversion of pepsinogen to pepsin at pH < 2. Peptic activity is then calculated from the quantity of tyrosine-containing peptide fragments liberated by digestion.

Faecal egg counts were estimated using a modified McMaster Technique where each egg counted represented 50 eggs/g (Appendix 7 SOP 1).

Worm counts were estimated (Appendix 7 SOP 6) using guidelines described by WAAVP (Wood *et al.* 1995). After slaughter by captive bolt, the recovered gastrointestinal tract from each deer was separated into different organs (abomasum, small intestine and large intestine) and frozen at -20°C until further processed. Individual organs were later thawed and thoroughly washed to a measured volume. A 5% aliquot was removed and sieved through a 37.5 μm sieve. In addition, the abomasal tissue was subject to digestion in pepsin+HCL at 37°C until the mucosal tissue was disrupted, then 5% aliquots of these were also counted. The parasites present were counted using a stereo microscope at 40X magnification.

For the identification of the species, male worms (up to 50) in the abomasum and small intestine and adults (females and males) in the large intestine were recovered and fixed in 70% alcohol to identify to species. If less than 50 males in the abomasum and small intestine were recovered, then all available male nematodes were examined. In addition, the abomasum and large intestine were subject to a pepsin digest to recover immature larvae. This comprised a digest mixture of 0.4% Pepsin (Pepsin A, BDH 390324L) + 1.7%HCl with the tissue digested for 2 hours (Appendix 7 SOP 6).

To estimate establishment rates by genus in the abomasum the adult worm burdens were divided by the total number of larvae given up to 12 days before euthanasia. For *Oesophagostomum* spp. the establishment rates were estimated taking into account the number of doses up to 21 days before euthanasia.

2.2.5 Identification of Oesophagostomum sikae

Nematodes in the large intestine conforming to the morphological description of *O. sikae* (Popova 1965) were confirmed as the species present by PCR comparing it with the sequence described by Patrelle *et al*. (2014) and against *O. radiatum* from cattle in New Zealand. For that, three *O. sikae* of deer origin and four *O. radiatum* of cattle origin were lysed in a "lysis solution" for the DNA extraction and then the ITS-2 rDNA regions were amplified, cloned and sequenced as published by Bisset *et al.* (2014).

2.2.6 Statistical Analyses

All statistical analyses were carried out using SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, USA). Repeated measures of FEC, blood parameters, daily weight gain and voluntary feed intake on the same animals were analysed using the MIXED procedure with a linear mixed model. The model included the fixed effect of treatment, day, interactation of treatment-by-day and the random effect of animal to account for repeated measures. The residuals were assumed to be correlated with a compound symmetry covariance structure. Faecal egg counts and worm burdens were transformed by natural logarithm plus one, prior to analysis to normalize the data. Because the different groups were slaughtered at different times, comparisons between them were performed only where all treatment groups were present. Serum was available until Day 28 for HD and 29 for MD and LD but values from whole blood were only available until Day 22. Thereafter treatment groups were not compared as repeated measurements and were analysed by day. The least squares means (LSM) and standard errors were obtained and used for multiple comparisons using the least significant difference test as implemented in SAS. The LSM for log (FEC+1) and log (worm burden+1) were presented as back-transformed LSM with 95% confidence intervals.

2.3 Results

2.3.1 Faecal Egg Counts

A summary of LSM of faecal egg counts until Day 29 for all treatment groups is presented in Figure 0.1. The faecal egg counts in the Control Group were constantly zero. Until Day 29, there was a significant difference in LSM egg count between treatment group (p=0.002), day (p <0.0001) and an interaction between them (p<0.001).

Significant differences started from Day 22 where the HD Group had higher egg counts than the LD (p< 0.0001) and MD (p < 0.001) groups. At Day 29 all groups were different from each other: HD > MD (p=0.025), HD > LD (p<0.0001), MD > LD (p=0.027).

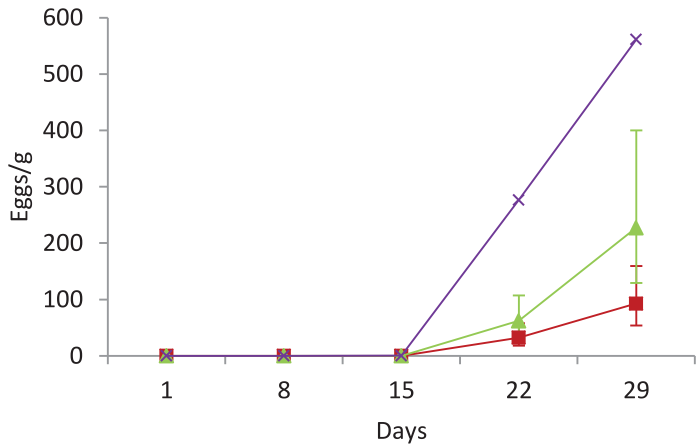


Figure 0.1 Least square means and 95% confidence interval of faecal egg counts from Day 1 (first treatment dose) to Day 29 according to Low Dose, Medium Dose and High Dose groups.

2.3.2 Worm Counts

Data are presented in Table 0.3 to Table 0.6.

Table 0.3 Least square means (and 95% confidence interval) of abomasal nematode counts

Group	*Ostertagia*-type LSM (CI)	*Haemonchus* sp. LSM (CI)	*Trichostrongylus* spp. LSM (CI)	Larvae LSM (CI)
HD	9686 (8594- 10915)	173 (87- 343)	0 (-1- 0)	1896 (1348- 2668)
MD	6029 (5350- 6795)	80 (40- 159)	0 (0- 1)	579 (412- 816)
LD	2227 (1976- 2510)	30 (15- 60)	0 (-1- 0)	80 (56- 113)

HD (High Dose Group), MD (Medium Dose Group) and LD (Low Dose Group).

Abomasum: Four species of the subfamily Ostertaginae were recovered from the treated deer. The order of frequency was *S. spiculoptera* > *S. asymmetrica* > *O. leptospicularis* (including *O. kolchida*) > *T. circumcincta* as presented in Table 0.4. Too few *Trichostrongylus* adults were found to be able to identify which species were present.

Table 0.4 Least square means (and 95% confidence interval) of the identified *Ostertagia*-type nematodes.

Group	*S. spiculoptera* LSM (CI)	*S. asymmetrica* LSM (CI)	*O. leptospicularis* LSM (CI)	*T. circumcincta* LSM (CI)
HD	5582 (4776- 6523)	1104 (487- 2499)	1689 (1222- 2336)	1 (0- 6)
MD	3361 (2876- 3928)	1579 (697- 3575)	927 (671- 1282)	1 (0- 6)
LD	1200 (1027- 1402)	634 (280- 1437)	308 (223- 426)	1 (0- 5)

HD (High Dose Group), MD (Medium Dose Group) and LD (Low Dose Group).

Table 0.5 Least squares means (and 95% confidence interval) of *Oesophagostomum* spp. worm counts in the large intestine**.**

Group	*Oesophagostomum* spp. LSM (CI)	Larvae LSM (CI)	*O. venulosum* LSM (CI)	*O. sikae* LSM (CI)
HD	1784 (1236-2575)	2701 (87- 343)	1810 (1227- 2671)	1 (0- 5)
MD	923 (639-1332)	1371 (15- 60)	885 (610- 1282)	12 (4- 36)
LD	582 (403- 841)	450 (40- 159)	577 (398- 836)	1 (0- 6)

HD (High Dose Group), MD (Medium Dose Group) and LD (Low Dose Group).

Small Intestine: there were very few nematodes in any group. There were negligible numbers of *Trichostrongylus (T. colubriformis* and *T. vitrinus)* and *Cooperia (C. punctata, C. pectinata)* in all the treatment groups.

Large Intestine: Two species were recognized: *O. venulosum* was the predominant species with a small proportion of *Oesophagostomum sikae* also being identified (Patrelle *et al.* 2014). No additional adult or larval nematodes were recovered after pepsin/HCl digestion of the large intestine. Numerous inflammatory nodules in the mucosa of the terminal small intestine and large intestine together with oedema and a general inflammatory response in the large intestine were observed (examples presented in Figure 0.2). Examination of the species of *Oesophagostomum* revealed the predominance of *O. venulosum* but some adult *O. sikae* were also recovered (Table 0.5)

Figure 0.2 Large intestine and small intestine nodules and oedema from deer in the Medium Dose Group

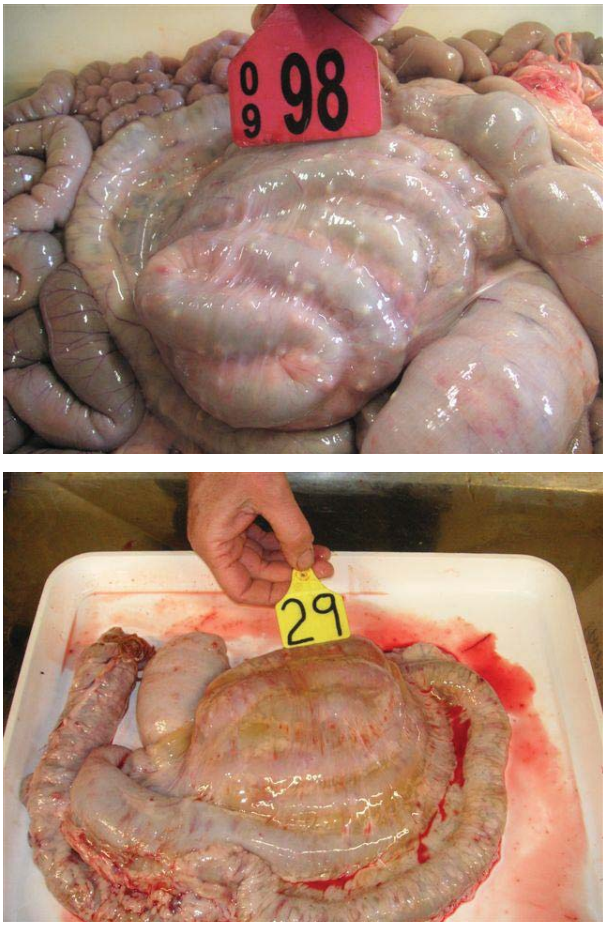


Table 0.6 Number of doses, total number of larvae administered, and establishment rate (% worms counted/total larval dose until 12 days before slaughter), for each abomasum genera in each group.

Group	Nº doses until 12 days before slaughter	Total larvae given			Establishment rate (%)		
		Ostertagia-type (range)	*Haemonchus* spp. (range)	*Trichostrongylus* spp. (range)	*Ostertagia*-type	*Haemonchus* spp.	*Trichostrongylus* spp.
HD	8	20000 (19000-21000)	336 (0-504)	3000 (1500-4000)	48 (32-59)	51 (18-107)	0 (0-0)
MD	9	13500 (12825-14175)	225 (0-338)	2025 (1013-2700)	45 (31-56)	36 (0-89)	0 (0-1)
LD	12	6000 (5700- 6300)	96 (0-144)	900 (450-1200)	37 (25-55)	31 (0-125)	0 (0-2)

HD (High Dose Group), MD (Medium Dose Group) and LD (Low Dose Group).

Table 0.7 Number of doses, total larvae administered and establishment rate (%, No worms counted/total larval dose until 21 days before slaughter) of *Oesophagostomum* spp.

Group	Nº of doses until 21 days before slaughter.	Total larvae (range)	Establishment rate (%)
		Oesophagostomum spp.	
HD	4	13332 (12998-13499)	13 (8-23)
MD	5	10000 (9750-10125)	9 (1-19)
LD	8	5336 (5203-5403)	11 (5-18)

HD (High Dose Group), MD (Medium Dose Group) and LD (Low Dose Group).

2.3.3 Blood parameters

1.5.2.5 Albumin Levels

A summary of LSM of albumins levels until Day 29 for all treatment groups is presented in Figure 0.3. Up to Day 29, when the first deer required euthanasia, repeated measures showed a significant difference between treatments ($p<0.001$), day ($p<0.0001$) and the interaction between treatment and day ($p<0.0001$). Overall, the Control Group had significantly higher serum albumin values than the MD Group ($p<0.01$) and the HD Group ($p=0.001$) but not the LD group ($P>0.05$). The LD Group also had significantly higher serum albumin values than the HD Group ($p=0.009$).

On Day 29, the Control Group had significantly higher serum albumin values than the LD Group ($p=0.022$), the MD Group ($p<0.0001$) and the HD Group ($p<0.0001$). The LD Group had significantly higher serum albumin than the MD Group ($p<0.0001$) and the HD Group ($p<0.0001$). The MD Group was not significantly different to the HD Group ($p=0.078$).

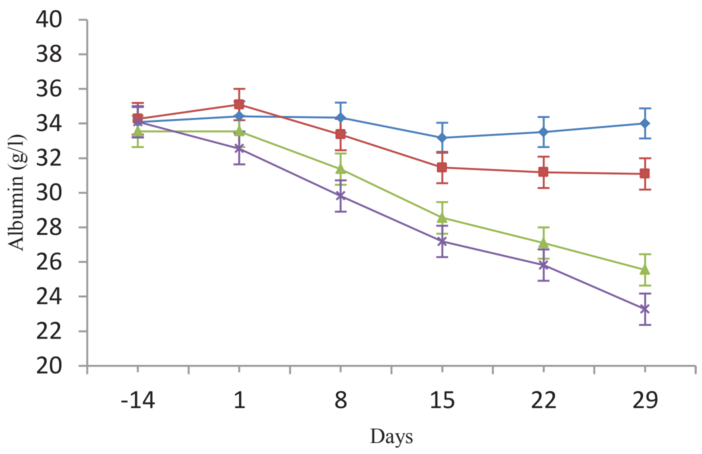


Figure 0.3 Least square means (± SE) of serum albumin values (g/l) from Day -14 (pre-treatment) to Day 29 for the Control , Low Dose, Medium Dose and High Dose groups.

1.5.2.6 Globulin Levels

A summary of LSM of globulin levels until Day 29 for all treatment groups is presented in Figure 0.4. Until Day 29, repeated measures showed a significant difference between treatments ($p<0.0001$), day ($p<0.0001$) and the interaction between treatment and day ($p<0.0001$). Overall, the Control Group had significantly lower serum globulin values than the LD Group ($p=0.006$), MD Group ($p<0.0001$) and the HD Group ($p<0.0001$).

At Day 29, the Control Group had significantly lower serum globulin levels than the LD Group ($p<0.001$), the MD Group ($p<0.0001$) and the HD Group ($p<0.0001$). The LD Group was marginally not significantly different than the MD Group ($p=0.06$).

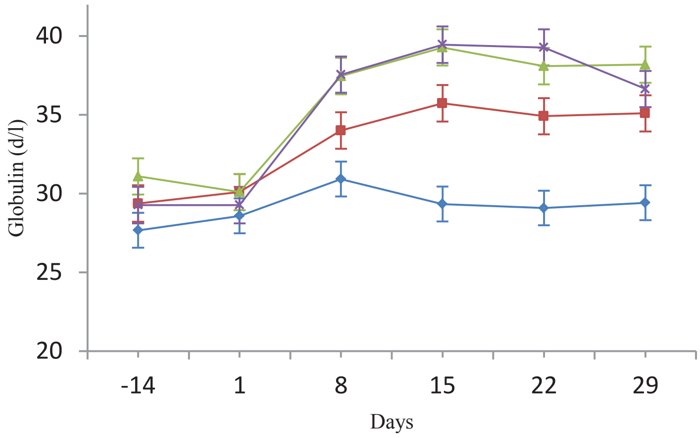


Figure 0.4 Least square means (± SE) of serum globulin values (g/l) from Day -14 (pre-treatment) to Day 29 for the Control , Low Dose , Medium Dose and High Dose groups .

1.5.2.7 Serum albumin to Globulin Ratios

A summary of LSM of the serum albumin to globulin ratios until Day 29 for all treatment groups is presented in Figure 0.5. Until Day 29, repeated measures showed a significant difference between treatments ($p<0.0001$), day ($p<0.0001$) and the interaction between treatment and day ($p<0.0001$). Overall, the Control Group had significantly higher serum albumin to globulin ratio than the LD Group ($p=0.002$), MD Group ($p<0.0001$) and the HD Group ($p<0.0001$). The LD Group also had significantly higher serum albumin to globulin ratio than the MD Group ($p=0.006$) and the HD Group ($p=0.009$).

At Day 29, the Control Group had significantly higher albumin to globulin ratio than the LD Group ($p<0.0001$), the MD Group ($p<0.0001$) and the HD Group ($p<0.0001$). The LD Group had significantly higher albumin to globulin ratio than the MD Group ($p<0.0001$) and the HD Group ($p<0.0001$).

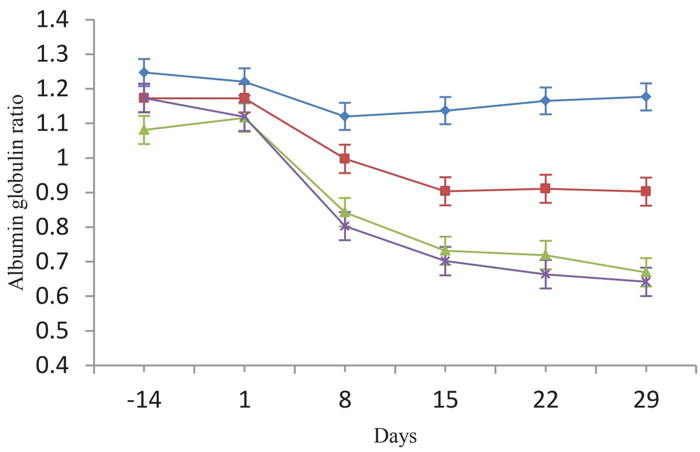


Figure 0.5 Least square means (±SE) of serum albumin to globulin ratios from Day -14 (pre-treatment) to Day 29 for the Control, Low Dose, Medium Dose and High Dose groups.

1.5.2.8 Eosinophil counts

A summary of LSM of eosinophil counts until Day 22 for all treatment groups is presented in Figure 0.6. Until Day 22, repeated measures showed a significant difference between treatments ($p<0.001$), day ($p<0.0001$) and the interaction between treatment and day ($p<0.0001$). Overall, the Control Group had significantly lower eosinophils counts than the LD Group ($p<0.001$), MD Group ($p=0.0001$) and the HD Group ($p<0.001$).

At Day 22, the Control Group had significantly lower eosinophil counts than the LD Group ($p<0.0001$), the MD Group ($p<0.0001$) and the HD Group ($p=0.007$).

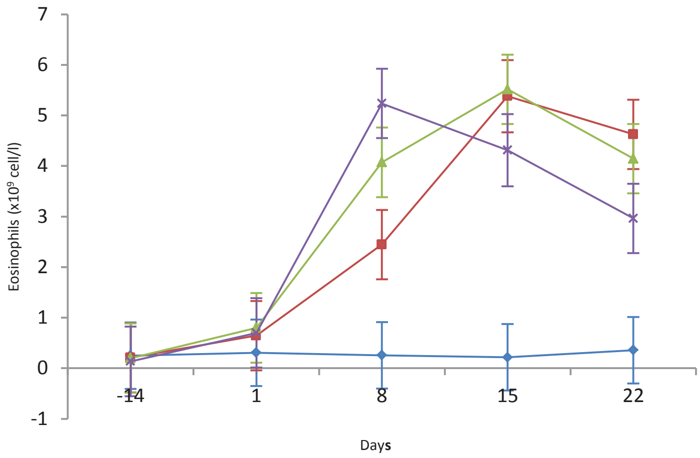


Figure 0.6 Least square means (±SE) of eosinophil counts (x10^9 cells/l) from Day -14 (pre-treatment) to Day 22 for the Control, Low Dose, Medium Dose and High Dose groups.

1.5.2.9 Basophil counts

A summary of LSM of the serum basophil counts until Day 22 for all treatment groups is presented in Figure 0.7. Until Day 22, repeated measures showed a significant difference between treatments (p=0.006), day (p<0.0001) and the interaction between treatment and day (p<0.0001). Overall, the Control Group had significantly lower basophil counts than the LD Group (p=0.03) and the HD Group (p<0.001). Also, the MD Group had significantly lower basophil counts than the HD Group (p=0.03).

At Day 22, the Control Group had significantly lower basophil counts than the LD Group (p<0.0001), the MD Group (p=0.001) and the HD Group (p<0.0001). The HD Group had significantly higher basophil counts than the LD Group (p=0.037) and the MD Group (p=0.004).

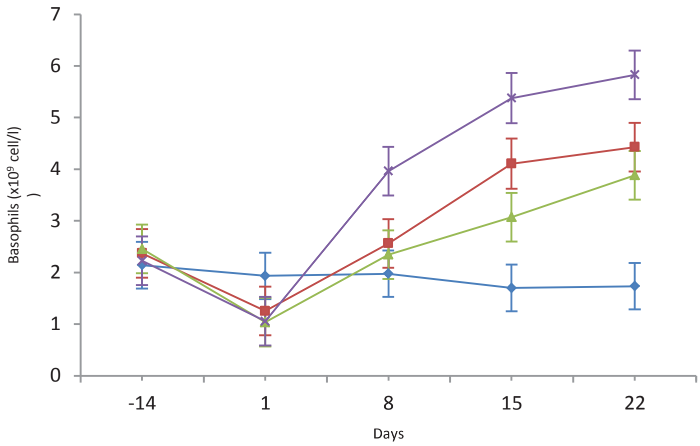


Figure 0.7 Least square means (±SE) of basophil counts (x10^9 cells/l) from Day -14 (pre-treatment) to Day 22 for the Control , Low Dose , Medium Dose and High Dose groups .

1.5.2.10 Other blood parameters

No significant differences (p>0.05) between treatment groups were seen for neutrophils, lymphocytes, monocytes, red blood cells, haemoglobin, packed cell volume, pepsinogen levels, or the interaction between treatment and day of trial for each parameter.

2.3.4 Liveweight gain

A summary of LSM of the liveweight gains until Day 22 for all treatment groups is presented in Figure 0.8. Until Day 22 repeated measures showed a significant difference between treatments (p<0.0001), intervals between days (p<0.0001) and the interaction between treatment and intervals between days (p<0.0001). Overall, the Control Group had significantly higher liveweight gains than the MD Group (p<0.01) and the HD Group (p<0.0001). Also, the LD Group had significantly higher liveweight gains than the MD Group (p=0.03) and the HD Group (p<0.001).

In the interval between Day 15 and Day 22, the HD Group had significantly lower liveweight gains than the Control Group (p=0.025), the LD Group (p=0.003) and the MD Group (p=0.022).

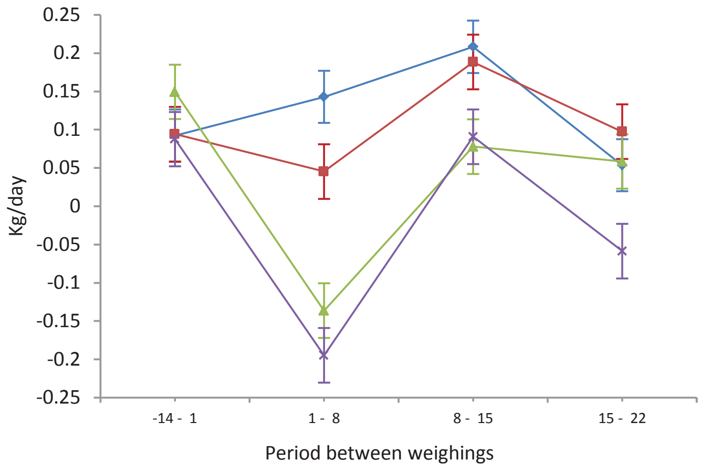


Figure 0.8 Least square means (±SE) of the daily growth rate (kg/day) from the interval Day -14 (pre-treatment) to Day 1 (first treatment dose) up to the interval Day 15 to Day 22 for the Control , Low Dose , Medium Dose and High Dose groups .

2.3.5 Voluntary Feed intake

A summary of LSM of the crude individual voluntary feed intake until Day 27 for all treatment groups is presented in Figure 0.9. Until Day 27, repeated measures showed a marginally non-significant difference between treatments (p=0.051), but significant difference between days (p<0.0001) and significant interaction between treatment and days (p<0.0001). Overall, the HD Group had a significantly lower feed intake than the Control Group (p=0.019) and the LD Group (p=0.024).

At Day 27, the Control Group had a significantly higher feed intake than the MD Group (p<0.001) and the HD Group (p<0.0001). The LD Group had significantly higher feed intake than the MD Group (p<0.001) and the HD Group (p<0.0001).

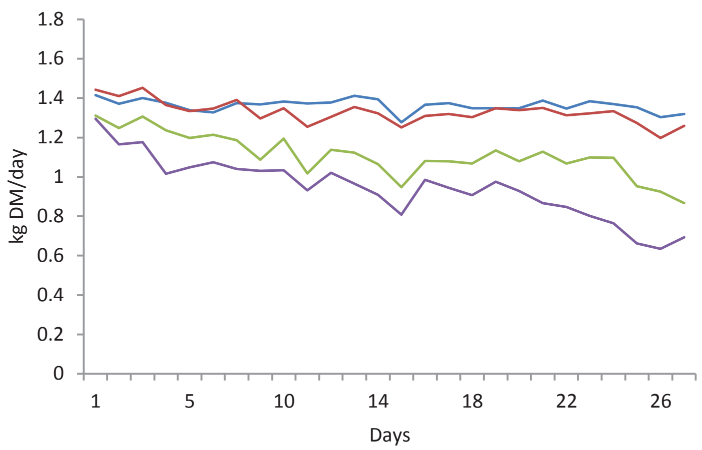


Figure 0.9 Least square means of daily intake (kg/head/day) from Day 0 (1 day pre-treatment) to Day 27 for the Control ——, Low Dose ——, Medium Dose —— and High Dose ——groups

Feed intake was rapidly reduced over the first weeks of infection in the HD Group, and was significantly different from the Control groups from Day 4 onwards (p=0.004). Significant differences between the MD Group and the Control Group were observed from Day 9 (p=0.021).

There were no differences between the Control Group and the LD Group (p>0.05).

2.4 Discussion

The aim of this trial was to investigate the impact of GIN on food intake, weight and haematological and biochemical markers, with a focus on the *Ostertagia*-type nematodes because of their importance for deer. The infective dose used in this trial was intended to infect the deer with sufficient *Ostertagia*-type nematodes to cause an effect on growth rate but without overt clinical signs. The number of *Ostertagia*-type larvae given was similar to those used by Hoskin *et al*. (2000b) where an effect on growth rate was observed in the absence of clinical signs. In the present study the mixed dose of larvae also contained a high proportion of *Oesophagostomum* spp. larvae. Historically this was presumed to be *O. venulosum* as this has been the only *Oesophagostomum* species seen on the Massey University deer farm (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b). In this study *O. venulosum* was the predominant species but a small proportion of *O. sikae* were also identified. *O. sikae* has not previously been recognised in New Zealand although it does morphologically resemble *Oesophagostomum radiatum* which has been reported once previously (McKenna 1999). Hence, while some aspects of this study were terminated early and therefore the objectives were not fully met, despite achieving significant results, this study yielded important novel observations of clinical and production significance to deer, relating to the pathogenicity of *Oesophagostomum* species.

The onset of clinical signs at Day 22 in the HD treatment group followed by the MD Group was most likely due to the occurrence of numerous inflammatory nodules in the mucosa of the terminal small intestine and large intestine together with substantial oedema and a general inflammatory response around the large intestine. The first report of *O. radiatum* in deer in New Zealand, which was likely to be misidentified as *O. sikae*, did not mention if nodules were seen (McKenna 1999). It is generally accepted that *O. venulosum* is a non-pathogenic parasite and does not induce nodule formation in natural infections. However, in previous experiments with heavy infections in sheep it has induced the formation of nodules together with diarrhoea (Goldberg 1952; Clark *et al.* 1978). It is unknown if *O. sikae* induced the nodule formation, but it may have contributed as nodules have never been reported in the large intestine of deer previously with *O. venulosum.*

The measured establishment rate of *Oesophagostomum* spp. was consistent between the three infected groups but not as high as would be expected commensurate with the damage. However, the establishment rate recorded was likely lower than in reality because larvae, which likely contributed to the tissue pathology, could not be counted. It was intended that the larvae would be recovered from the mucosa of the large intestines with pepsin digestion, but this procedure was not effective at releasing the larvae. The minimum prepatent period for *O. venulosum* is 28-31 days with the larvae within the mucosa for the first 4 days before emerging as L4 and then maturing (Anderson 2000). *O. radiatum* spend 7-14 days within the mucosa before emerging as L4 larvae and continuing to mature (Anderson 2000). Assuming *O. sikae* is similar to *O. radiatum,* it is plausible that many of the larvae given to these deer may still be within the mucosa. There are no previous reports as to whether *O. sikae* induce nodule formation but given the findings here it seems likely that they contributed to the gross pathology and clinical signs seen.

For other types of GIN the overall establishment rates were similar between the treatment groups. In sheep and cattle, immature adult *Ostertagia*-type nematodes are expected to emerge from the lumen of the abomasum between 7 and 10 days after infection (Wood *et al.* 1995). However, for species in deer this information is not available. Therefore, to calculate the establishment rate of *Ostertagia*-type species in deer the timing of development in other ruminants was extrapolated to deer. The establishment rates in these young deer were higher (37% - 48%) than establishment rates of *T. circumcincta* (23.4%) in repeated experimental infections in sheep (Gaba *et al.* 2006). This may have occurred because the establishment rate can vary depending of the immunological response of the host and the virulence of the parasite and in this study another host species and another nematodes species were being investigated.

For *H. contortus* the immature adult parasites are present from about 9 to 11 days after infection (Wood *et al.* 1995). The establishment rate for *Haemonchus* sp. larvae observed in this study (31-51%) was also higher than that observed in another study in which young deer were infected with a single dose of sheep gastrointestinal nematodes and where the establishment rate only reached 10% (Tapia-Escárate *et al.* 2015b; Chapter 5). An explanation for this difference could be that *H. contortus* of deer origin can establish more successfully in deer than *H. contortus* of sheep origin. Nevertheless, both results suggest that this species could establish clinically significant burdens in deer.

The establishment rate for *Trichostrongylus* was particularly low and the reason for this is not apparent as *T. axei* in particular has been found to be present in other studies (Chapters 4, 5 and 6) in at least modest numbers. Only one *T. askivali* was observed in one deer.

The blood and serum biochemistry results reflect a classic response to parasite challenge related to damage to the gut with a rapid turnover rate of cells and a host immune response. There was a marked serum albumin decrease and a globulin increase over the course of the study with a clear relationship to the dose rate. Therefore, as expected, over time there was a decrease in the albumin:globulin ratio. This has been associated with *O. ostertagi* in cattle (Murray *et al.* 1970) and with *T. circumcincta* in sheep (Coop *et al.* 1982), but also with heavy infections with *O. venulosum* in sheep (Badrie and Kamenov 1982). In addition, again associated with the immune response to parasitic infection, there was a similar increase related to dose rate in the eosinophil and basophils counts. It is well known that eosinophils and basophils are associated with an immune response against helminth infections mostly linked with Type 2 immune responses. Both of these cell types have cytoplasmic granules that are released upon activation and they can also secrete IL4 and IL13 that contribute to the activation of genes that mediate immunity against helminths and ticks (Voehringer 2016).

Serum pepsinogen values showed no significant difference between treatments or the interaction between treatment and day of trial. There have only been a few studies that have measured pepsinogen levels in deer. Audigé *et al*. (1998) observed that pepsinogen values had an inverse relationship at the herd level when related to the summer growth of weaner deer, but this relation was not significant at the individual level. In addition, Hoskin *et al*. (2000b) found a correlation between burdens of *Ostertagia*-like nematodes and pepsinogen values, whilst others were not able to show a relationship with burdens of these same nematode species (van der Heide 2009). Therefore, is it not surprising that even with higher burdens of *Osteragia*-type nematodes in this study, no differences were found between groups.

The differences between treatment groups for voluntary feed intakes show a tendency of a decrease with a "dose-rate" response. This was evident when comparing the HD group against the Control and the LD treatment groups.

Over the whole period the Control and the LD group had a higher liveweight gain than the MD and the HD treatment groups. It is commonly accepted that gastro-intestinal parasitism reduces voluntary feed intake and the efficacy of food utilization (Coop and Holmes 1996). Previous studies in deer have also demonstrated a reduction in voluntary feed intake and liveweight gain associated with subclinical infection of parasitic nematodes (Hoskin *et al.* 2000b; Hoskin *et al.* 2007). In the present study the decrease in liveweight gain and voluntary feed intake was most likely influenced by the both the number and range of species in the continuous challenge with larvae, together with the pathology which occurred in the large intestine and terminal small intestine. However, the pattern of liveweight gain is not easily explained by feed intake.

Overall this study did not achieve the principal aim of investigating the relative pathogenicity of *Ostertagia*-type parasites as the animals were clinically affected by the large intestinal lesions associated with *Oesophagostomum* spp. nematodes.

Nevertheless, the latter finding was a potentially important novel finding from this study. Further studies will be necessary to fully assess the pathogenicity of *O. sikae* as well as to assess more clearly the impact of *Ostertagia*-type nematodes. Data of weight and food intake, along with haematological and biochemical markers for parasitism were consistent with other studies in other host species and add to the overall knowledge of the interaction between the host and gastrointestinal parasites in deer.

2.5 Acknowledgements

This study was funded by AgResearch Ltd New Zealand. The study was performed at the Massey University Deer Unit on stags purchased from a commercial farm in Wanganui. We acknowledge the large input of time and effort of Geoff Purchas in daily feeding, care, management and handling of deer. We thank Jenny Nixey and her vet nursing students for their help with data collection. Thanks to Barbara Adlington for her culture work in the laboratory and Anne Tunnicliffe, Sarah Pain, Ben Bauer, and visiting students for helping with sample handling. Thank you also to Tiare Delaune and Marjorie Turlin for helping with adult worm counts. The tests of various blood parameters were performed at New Zealand Veterinary Pathology (Massey University) and the staff involved are thanked for their assistance.

Chapter 3 Analysis of the pathogenicity and diagnosis of GI nematode infections in young farmed red deer – Supplementary follow-up study

3.1 Introduction

The initial study (Chapter 2) was the first study involving infections with only gastrointestinal nematodes in red deer. That study was terminated earlier than planned due to the onset of clinical signs resulting from the occurrence of numerous inflammatory nodules in the mucosa of the terminal small intestine and large intestine together with substantial oedema and a general inflammatory response around the large intestine caused by *Oesophagostomum* sp. The aim of this follow-up study was to investigate the impact of artificial infection with a lower dose of deer-origin mixed species of larvae of gastrointestinal nematodes than used in that study. It made use of the control animals from the Initial Study which were then 57 days older at the beginning of this supplementary trial.

3.2 Materials and methods

3.2.1 Experimental Design.

The study design involved 12 deer (the Control Group in the Initial Study described in Chapter 2) which were allocated into two groups of six animals each, housed in two groups of three per pen with all animals in the same pen being in the same treatment group. One group were trickle-infected three times per week with 30% of the number of larvae given to the low dose of the Initial Study (Lower Dose, designated LrD) and with the same source of larvae (see Section 2.2.3). The other group remained as the uninfected Control Group. Deer were infected over 44 days (=18 occasions) and then euthanased. Table 0.1 shows the schedule of events for the trial.

Table 0.1 Schedule of events.

Days	Event
-8 - 0	Each group randomly assigned to treatment. Initial voluntary feed intake (VFI) measured, faecal samples and initial blood samples obtained. Deer weighed.
1 - 43	Trickle infection 3x per week. Weekly measurement of weights, faecal and blood samples and group VFI.
44	Deer euthanased for worm counts.

3.2.2 Animals and management

The history of these deer is the same as in the Initial Study except they were 57 days older. In brief, they were effectively treated with an anthelmintic on arrival, prior to housing and then remained free of nematodes during the Initial Study and then into this Supplementary Study. The full description of feeding, management, euthanasia, necropsy and recording is described in Chapter 2.

3.2.3 Source of Larvae

The larvae (Table 0.2) were from the same pool as used in the Initial Study and had been stored at 10ºC since they were cultured.

Table 0.2**:** Treatment regime showing number of larvae of each species per individual dose given 3 times per week and the total number of larvae during the whole period.

Dose of *Ostertagia*-type L3		Dose of *Oesophagostomum* spp. L3		Dose of *Haemonchus* sp. L3		Dose of *Trichostrongylus* spp. L3	
Individual (range)	Total (range)	Individual (range)	Total (range)	Individual (range)	Total (range)	Individual (range)	Total (range)
150 (143-158)	2700 (2565-2835)	200 (195-203)	3600 (3510-3645)	3 (0-4)	45 (0-68)	23 (11-30)	405 (203-540)

3.2.4 *Measurements*

Liveweight gains were measured weekly and voluntary feed intake (VFI=Offered-Rejected) was measured by pen (3 animals/pen) on a daily basis. To crudely estimate individual voluntary feed intake the feed consumption per pen was divided by the number animals in that pen. Blood samples were collected into plain and EDTA vacutainers via jugular venepuncture while deer were physically restrained. The following blood parameters were measured once a week during the study: total serum protein, serum albumin and globulin, white blood cell counts (including individual components; neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cells, haemoglobin and packed cell volume. These analyses were undertaken by a commercial laboratory (New Zealand Veterinary Pathology) using equipment calibrated for deer samples. Pepsinogen concentrations were determined using a method that has been validated previously (Scott *et al.* 1995) with minor modifications (Simcock *et al.* 1999).

Faecal egg counts were estimated using a modified McMaster Technique where each egg counted represented 50 eggs/g (Appendix 7 SOP 1).

Worm counts were estimated (Appendix 7 SOP 6) using guidelines described by WAAVP (Wood et al. 1995). After slaughter by captive bolt, the recovered gastrointestinal tract from each deer was separated into different organs (abomasum, small intestine and large intestine) and frozen at -20°C until further processed. Individual organs were later thawed and thoroughly washed to a measured volume. A 5% aliquot was removed and sieved through a 37.5 µm sieve. In addition, the abomasal tissue was subject to digestion in pepsin+HCL at 37°C until the mucosal tissue was disrupted, then 5% aliquots of these were also counted. The parasites present were counted using a stereo microscope at about 40X magnification.

For the identification of the species, male worms (up to 50) in the abomasum and small intestine and adults (females and males) in the large intestine were recovered and fixed in 70% alcohol to identify to species. If less than 50 males in the abomasum and small intestine were recovered then all available male nematodes were examined. In addition, the abomasum and large intestine were subject to a pepsin digest to recover immature larvae. This comprised a digest mixture of 0.4% Pepsin (Pepsin A, BDH 390324L) + 1.7%HCl with the tissue digested for 2 hours (Appendix 7 SOP 6).

To estimate establishment rates by genus in the abomasum the adult worm burdens were divided by the total number of larvae given up to 12 days before euthanasia. For *Oesophagostomum* spp. the establishment rates were estimated taking into account the number of doses up to 21 days before euthanasia.

3.2.5 *Statistical Analyses*

All statistical analyses were carried out using SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, USA). Repeated measures of FEC, blood parameters, daily weight gain and voluntary feed intake on the same animals were analysed using the MIXED procedure with a linear mixed model. FEC were transformed by natural logarithm plus one prior to analysis. The covariance of the blood parameters before the treatments with larvae, were also included in the model for the analysis. The least squares means (LSM) and standard errors were obtained and used for multiple comparisons using the least significant difference test as implemented in SAS. Faecal egg counts results were presented as back-transformed LSM with 95% confidence intervals.

Worm burdens were analysed after natural logarithmic transformation using the MIXED procedure with a linear mixed model. The LSM and confidence interval were obtained and used for multiple comparisons using the least significant difference test as implemented in SAS. Results were presented as back-transformed LSM with 95% confidence intervals.

3.3 Results

3.3.1 Faecal Egg Counts

A summary of LSM of faecal egg counts is presented in Figure 0.1. The faecal counts in the Control Group were constantly zero.

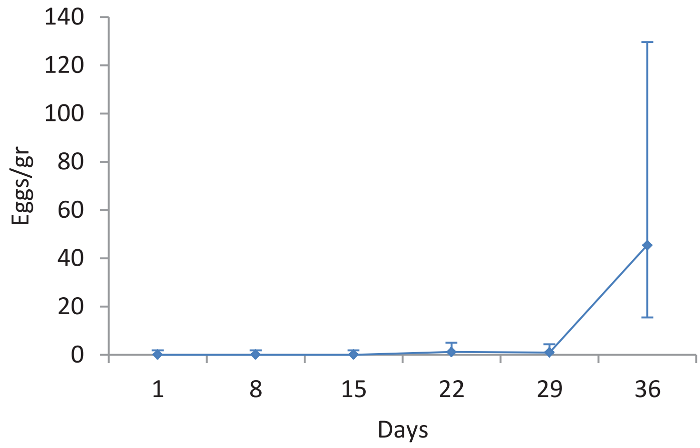


Figure 0.1 : Least square means and 95% confidence interval of faecal egg counts from Day 1 to Day 36 in the Control group .

3.3.2 Worm Counts

Abomasal worm counts are presented in Table 0.3

Table 0.3 Least square means (±95% confidence interval) of abomasal nematode counts.

Ostertagia-type LSM (CI)	*Haemonchus* sp. LSM (CI)	*Trichostongylus* spp. LSM (CI)	Larvae LSM (CI)
182 (109-302)	1 (0-3)	0 (0-0)	2 (0-15)

Abomasum: Three species of the subfamily Ostertaginae were recovered from the treated deer, in order of frequency *S. asymmetrica* > *O. leptospicularis* (including *O. kolchida*) > *S. spiculoptera* (Table 0.4).No significant differences were found between Ostertaginae species counts (p=0.087). No *Trichostrongylus* spp. adults were found.

Table 0.4 Least square means (±95% confidence interval) of the identified *Ostertagia*-type nematodes.

S. spiculoptera LSM (CI)	*S. asymmetrica* LSM (CI)	*O. leptospicularis* LSM (CI)
8 (1-51)	113 (64-199)	11 (1-86)

Table 0.5 Least squares means (±95% confidence interval) of total *Oesophagostomum* spp. worm counts in the large intestine, and counts for each species.

Oesophagostomum spp. LSM (CI)	*O. venulosum* LSM (CI)	*O. sikae* LSM (CI)
234 (165-330)	221 (167-294)	3 (0-27)

Large Intestine: The two species that were recognized in the initial study were also recognized in this one. Examination of the species of *Oesophagostomum* revealed the predominance of *O. venulosum* but some adult *O. sikae* were also recovered (Table 0.5).

Establishment rate: The establishment rates are given in Table 0.6 and Table 0.7.

Table 0.6 Number of doses, total number of larvae administered, and establishment rate (% worms counted/total larval dose until 12 days before slaughter), for each abomasum genera in each group.

Nº doses until 12 days before slaughter	Total larvae			Establishment rate (%)		
	Ostertagia- type (range)	*Haemonchus* spp. (range)	*Trichostrongylus* spp. (range)	*Ostertagia-* type (range)	*Haemonchus* spp. (range)	*Trichostrongylus* spp. (range)
14	2100 (1995-2205)	35 (0-53)	315 (158-420)	9 (5-14)	3 (0-9)	0 (0-0)

Table 0.7 Number of doses, total larvae administered and establishment rate (%, No worms counted/total larval dose until 21 days before slaughter) of *Oesophagostomum* spp.

Nº doses until 21 days before slaughter.	Total larvae given	Establishment rate (%)
	Oesophagostomum spp. (range)	*Oesophagostomum* spp. (range)
10	2000 (1950-2025)	12 (8-17)

3.3.3 Blood parameters

3.3.3.1 Albumin Levels

A summary of LSM of albumin levels is presented in Figure 0.2. Comparing the Control and the LrD treatment groups, repeated measures showed a non-significant difference between treatments (p=0.14), day (p=0.06) and the interaction between treatment and day (p=0.76). But significant differences were seen between the groups at day 0, before the first treatments with larvae.

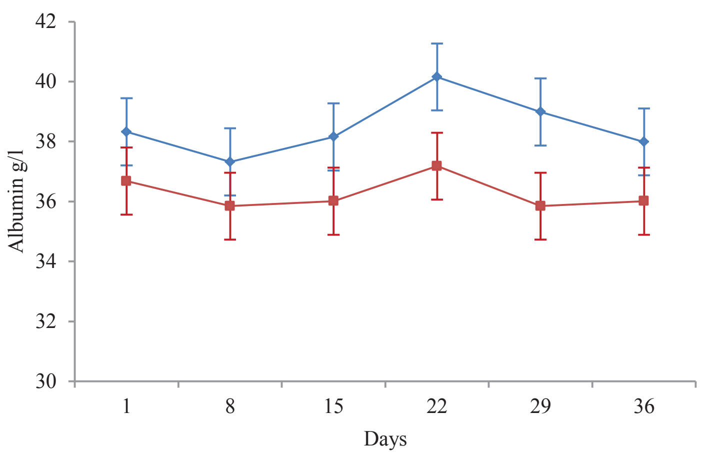


Figure 0.2: Least square means (± SE) of serum albumin values (g/l) from Day 1 to Day 36, for the Control and Lower Dose treatment groups.

3.3.3.2 Globulin Levels

A summary of LSM of globulin levels is presented in Figure 0.3. Comparing the Control, and the LrD treatment groups, repeated measures showed a significant difference between treatments (p=0.014), day (p<0.0001), the interaction between treatment and day (p<0.001) and also between the groups at day 0, before the first treatments with larvae (p=0.004).

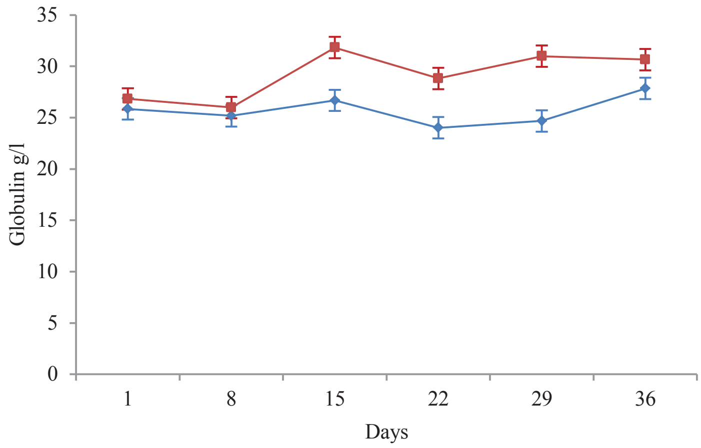


Figure 0.3: Least square means (± SE) of serum globulin values (g/l) from Day 1 to Day 36, for the Control and Lower Dose treatment groups.

3.3.3.3 Serum albumin to globulin ratios

A summary of LSM of serum albumin to globulin ratios is presented in Figure 0.4. Comparing the Control and the LrD treatment groups, repeated measures showed a significant difference between treatments (p=0.002), day (p=0.0001), the interaction between treatment and day (p=0.003) and also between the groups at day 0, before the first treatments with larvae (p=0.002).

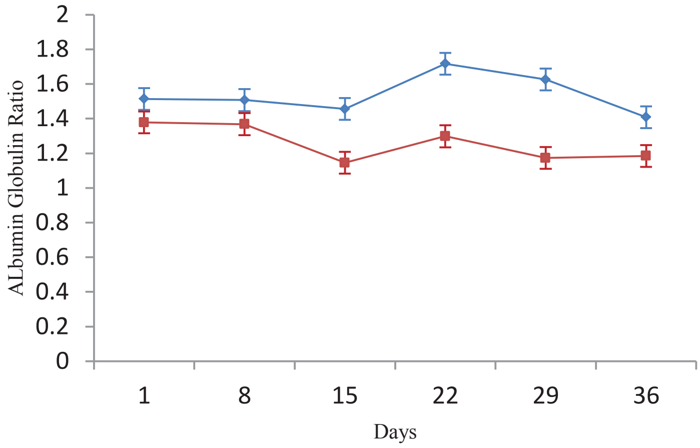


Figure 0.4: Least square means (± SE) of serum albumin globulin ratio from from Day 1 to Day 36 for the Control and Lower Dose treatment groups.

3.3.3.4 Eosinophil counts

A summary of LSM of eosinophil counts is presented in Figure 0.5. Comparing the Control and the LrD treatment groups, repeated measures showed a significant difference between treatments (p=0.0001), day (p<0.0001), the interaction between treatment and day (p<0.001), but non-significant between the groups at day 0, before the first treatments with larvae (p=0.144).

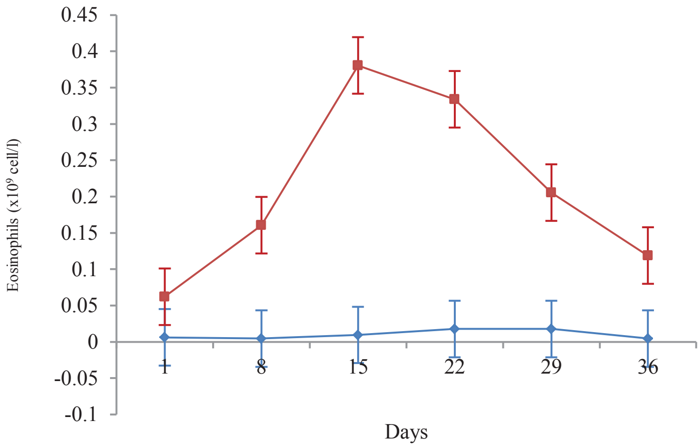


Figure 0.5: Least square means (± SE) of eosinophils counts (x10^9 cells/l) from from Day 1 to Day 36, for the Control and Lower Dose treatment groups.

3.3.3.5 Basophil counts

A summary of LSM of the serum basophil counts is presented in Figure 0.6. Comparing the Control and the LrD treatment groups, repeated measures showed a significant difference between treatments (p=0.029), day (p<0.001), the interaction between treatment and day (p<0.0001), and also between the groups at day 0, before the first treatments with larvae (p=0.021).

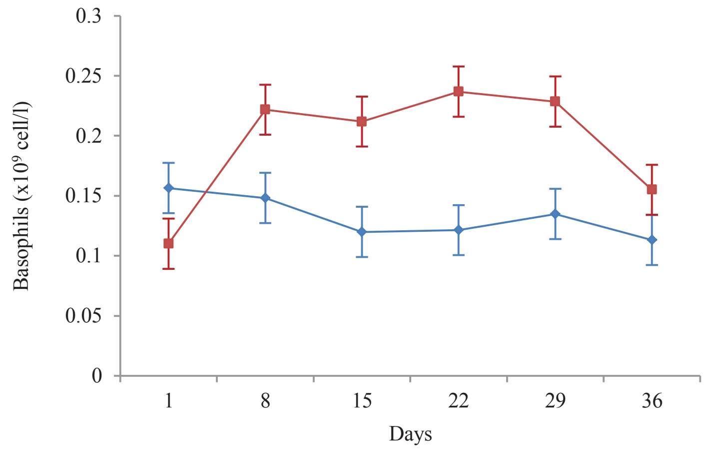


Figure 0.6: Least square means (± SE) of basophils counts (x10^9 cells/l) from Day 1 to Day 36, for the Control and Lower Dose treatment groups.

3.3.3.6 Other blood parameters

No significant difference was observed between the treatment and control groups for neutrophils, lymphocytes, monocytes, red blood cells, haemoglobin and packed cell volume values.

No significant difference between treatment and control groups was seen in the pepsinogen concentrations between treatments (p= 0.16) or the interaction between treatment and day (p=0.94).

3.3.4 Liveweight gain

A summary of LSM of the liveweight gain is presented in Figure 0.7. Comparing the Control and the LrD treatment groups, repeated measures showed no significant difference between treatments (p=0.27), period (p=0.33) or significant interaction between treatment and period (p=0.69).

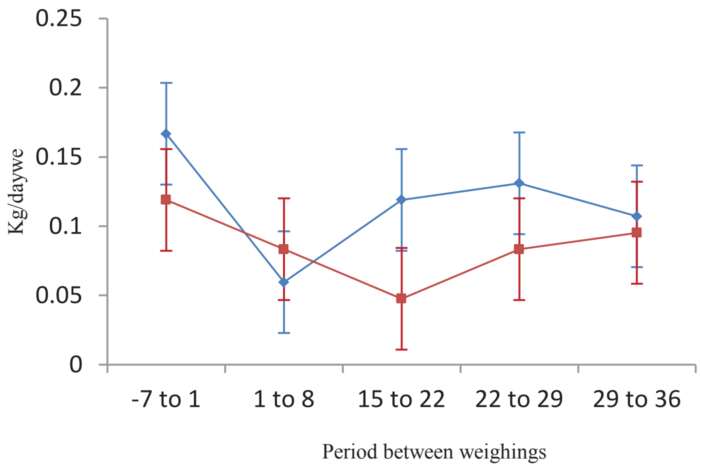


Figure 0.7: Least square means (± SE) of liveweight gain from from Day 1 to Day 36 for the Control and Lower Dose treatment groups.

3.4 Feed intake

A summary of LSM of the feed intake is presented in Figure 0.8. Comparing the Control and the LrD treatment groups, repeated measures showed a significant difference between days (p=0.001) but no significant difference between treatments (p=0.36) or significant interaction between treatment and period (p=0.17).

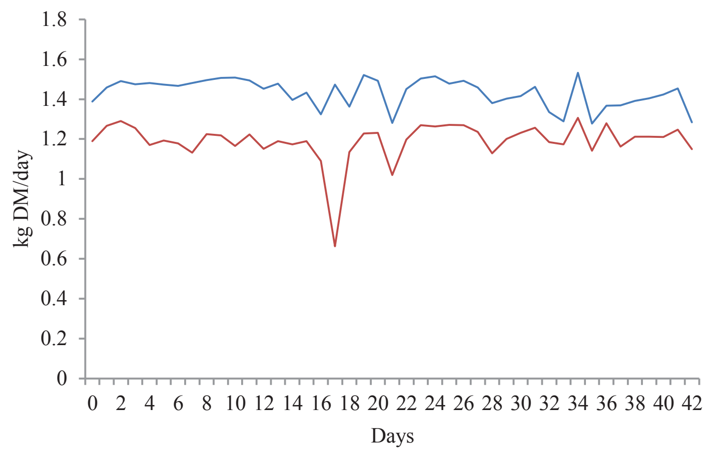


Figure 0.8: Least square means (± SE) of feed intake (kg DM/day) from Day 0 to Day 42, for the Control and Lower Dose treatment groups.

3.5 Discussion

As for the Initial Study the aim of this trial was to investigate the impact of gastrointestinal nematodes. The lower infective dose used in this follow-up trial was intended to infect the deer with sufficient GIN to cause an effect on growth rate but without overt clinical signs, since the doses used in the initial study did cause clinical signs, forcing a premature conclusion to that study. Hence, this study was an opportunistic use of the control animals from the initial trial, in an attempt to get more data to check for effects at a lower dose, but also to inform future challenge studies. In this study, there were no clinical signs or significant differences in voluntary feed intake or growth rates. This may be due to the use of older animals and/or lower dose compared with the Initial Study, but also the smaller sample size may have influenced the lack of statistical significance.

Even though it is not adequately controlled against the Initial Study and confounded by the age of the animals as well as the age of the larvae, the establishment rate in the Initial Study for *Ostertagia*-type species was 37% and for *Oesophagostomum* spp. was approximately 11% in contrast to 9% and 12%, respectively, found in this Supplementary Study. The lower establishment for the *Ostertagia*-type species in this study suggest that most likely the immune system of these animals was more developed and able to respond. It is known that age and previous exposure have an important effect on the immune response against nematodes (Sykes 1994). These deer had been exposed to infective larvae up until weaning which potentially provided some antigenic exposure to these parasites. Whether this was sufficient to stimulate an immune response despite being subsequently housed and not having continuous access to infective larvae is not known. Why there is an apparent difference between the establishment rates for *Oesophagostomum* and *Ostertagia*-types is unclear and suggests further study is required.

Of particular note was the observation that *Oesophagostomum sikae* was also present in this study but no nodules were observed, which suggests that the higher counts of *O. venulosum* in the Initial Study (Chapter 2) may be an important factor in the development of nodules seen in that study rather than *O. sikae* being responsible.

The Ostertaginae species recovered were only deer-specific species in contrast with the Initial study (Chapter 2) that additionally observed low burdens of *T. circumcincta*. The *Ostertagia*-type *S. asymmetrica* was the most common species found, in order of frequency, *S. asymmetrica* > *O. leptospicularis* > *S. spiculoptera*. However, as significant differences were not found between species, most likely because of the lack of statistical power, no comparison between the Initial pathogenicity study (Chapter 2) and this supplementary study can be performed.

There were few changes noted in the biochemistry. There was no obvious change in albumin levels but there was a significant interaction between treatment and day for globulin and for the albumin:globulin ratios which follows from this same change for globulin. This most likely reflects the immune response being triggered in these infected animals.

This lack of significant difference in the albumin levels in this chapter is consistent with there being no significant difference between the Low Dose group and the control group in the Initial Study (Chapter 2) which this may be explained by less damage and lower albumin losses through the mucosa.

Associated with the immune response to parasitic infection, there was a significant increase in the eosinophil and basophil counts in the infected animals. It's well known that eosinophils and basophils are associated with an immune response against helminth infections mostly linked with Type 2 immune response, involving redundant mechanisms. Both have cytoplasmic granules that are released upon activation and also can secrete IL4 and IL13 that contribute to the activation of genes that mediate immunity against helminth and ticks (Voehringer 2016).

Overall, the level of challenge in this follow-up study was less pathogenic than those of the Initial Study (Chapter 2). There were no inflammatory nodules observed in the large intestine which may be related to the age of these animals and their developed immune system as well as the lower larval dose.

3.6 Acknowledgements

This study was funded by AgResearch New Zealand. The study was performed at the Massey University Deer Unit on stags purchased from a commercial farm in Wanganui. We acknowledge the large input of time and effort of Geoff Purchas in daily feeding, care, management and handling of deer. We thank Jenny Nixey and her vet nursing students for their help with data collection. Thanks to Barbara Adlington for her culture work in the laboratory and Anne Tunnicliffe, Sarah Pain, Ben Bauer, and visiting students for helping with sample handling. Thank you also to Tiare Delaune and Marjorie Turlin for helping with adult worm counts. The tests of various blood parameters were performed at New Zealand Veterinary Pathology (Massey University) and the staffs involved are thanked for their assistance.

Chapter 4 Molecular identification and distribution of gastrointestinal nematodes in farmed red deer in New Zealand.

4.1 Introduction

In New Zealand, parasites have been acknowledged as a problem since the commencement of deer farming. Initially the most important parasite for deer was considered to be the lungworm which was originally believed to be *Dictyocaulus viviparus* (Watson and Charleston 1985) but is now known to be *Dictyocaulus eckerti* (Johnson *et al.* 2001a; Johnson *et al.* 2001b). However, new reports indicate an increase in the relative importance of gastrointestinal nematodes (GIN) which may be related to the rise of resistance to anthelmintics (Hoskin *et al.* 2005; Mackintosh *et al.* 2014b). Deer in New Zealand are infected by a relatively limited range of gastrointestinal species which are mainly concentrated in the abomasum including deer-specific nematodes in the sub-family Ostertagiinae (=*Ostertagia*-type; *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera* and *Ostertagia leptospicularis*) and also some GIN of sheep and cattle including; *Haemonchus contortus, Oesophagostomum venulosum, Teladorsagia circumcincta, Trichostrongylus axei, Trichostrongylus vitrinus, Nematodirus* spp.*, Chabertia ovina, Cooperia oncophora. Cooperia punctata*, *Cooperia pectinata* and *Cooperia curticei* (McKenna 2009a; Tapia-Escárate *et al.* 2015b; Chapter 5). In addition, *Oesophagostomum sikae* has been recently recognised in New Zealand (Chapter 2). This species is morphologically similar to *O. radiatum* and it is likely that earlier reports of *O. radiatum* in deer in New Zealand have misidentified *O. sikae*. Similarly *T. axei* was considered the only member of this genus in the abomasum of red deer in New Zealand, but *Trichostrongylus askivali* (McKenna 2009c) which is morphologically very similar, has also recently been recognized in New Zealand and its presence suggests it is not a recent arrival but it has been misidentified until this time.

Given the increased importance of GIN in deer it is important to review the occurrence of these nematodes. Traditional methods would involve the morphological identification of adult GIN to species level, which requires a deceased animal. It is more difficult to monitor the occurrence of GIN in live animals as measuring nematode egg counts combined with larval culture generally only allows identification to genus level. However, the recent development of molecular tools now allows larvae to be identified to species level. This has generally focused on the GIN from sheep and cattle where different techniques and protocols have been employed (Roos and Grant 1993; Callaghan and Beh 1994, 1996; Gasser *et al.* 2008; Bott *et al.* 2009; Roeber *et al.* 2012a; Roeber *et al.* 2012b; Demeler *et al.* 2013; Höglund *et al.* 2013; Roeber *et al.* 2013b; Bisset *et al.* 2014). The most commonly applied method is to identify regions of the first and the second internal transcribed spacer (ITS-1 and ITS-2) of rDNA as targets using PCR. These ITS regions are sufficiently conserved within species, yet different between species, to provide an accurate identification of different species.

The aim of this study was to investigate the occurrence of different GIN species in red deer in New Zealand. To achieve this objective protocols using PCR developed by Bisset *et al.* (2014) for identifying common sheep and cattle GIN were utilized. In addition, further protocols for some deer-specific GIN (Bisset unpublished) were used.

4.2 Materials and Methods

4.2.1 Samples

3.2.5.1 Farms and animals

Opportunistic use was made of samples collected primarily for a multi-species study of the epidemiology of Johne's disease (Heuer *et al.* 2012; Verdugo *et al.* 2012). Faecal samples were used from 59 farms around New Zealand. The North Island farms (n=25) were in the Manawatu-Wanganui region (n=14), Hawkes Bay (n=5), Bay of Plenty (n=2), Waikato (n=2), Gisborne (n=1) and the East Coast (n=1). The South Island farms (n=30) were in the Canterbury region (n=19), followed by Southland (n=7), Otago (n=4) and the West Coast (n=4). Deer were sampled per rectum using a new glove for each animal. The samples were collected from June 2009 to Sept 2010. The majority of the farms were sampled in winter (n=33), followed by spring (n=17), autumn (n=7) and summer (n=2). Deer of both sexes between 12 and 24 months of age were selected by choosing the first 20 moving through a race. In addition, some animals suspected of having clinical Johne's disease on some farms were sampled. Although the intent was to utilize the faecal samples of 20 sampled animals per farm, on some properties fewer were available, in which case all available faecal samples were used. Consequently, the number of samples ranged from 6 to 26 per farm, with an average of 19 sampled deer per farm.

To facilitate analysis, as the samples were not evenly distributed by season, they were divided into those collected in a "mild" season, (= spring, summer and autumn; n=26) or a "cold" season (= winter; n=33) groups. The farms were also stratified by farming type being those which were exclusively deer farms (D; n=16), those which co/cross grazed with sheep (DS; n=7) or cattle (DC; n=10) and those farms with all three host species (DCS; n=26).

3.2.5.2 Larval culture

Faecal samples from each farm were pooled and mixed with deionised water plus vermiculite and incubated between 23-25°C for 10 days. The mixtures were then placed in Baermann funnels and the active larvae were collected 24 hours later from the bottom of the funnel (Appendix 7, SOP 5). The larvae were stored at 10°C until selected for DNA extraction. From each farm 24 larvae were randomly selected. Overall, a total of 1416 larvae were collected for analysis.

4.2.2 PCRs

For the identification of larvae, primers developed by Bisset *et al.* (2014) for the identification of GIN found in sheep were utilised. These included *Teladorsagia circumcincta, Trichostrongylus axei, Haemonchus contortus, Trichostrongylus colubriformis; Trichostrongylus vitrinus, Cooperia curticei, Chabertia ovina, Oesophagostomum venulosum, Nematodirus spathiger, Cooperia oncophora* and *Ostertagia leptospicularis.* This study commenced as the work by Bisset *et al*. (2014) was continuing and the choice of primers used was not that finally recommended by these authors. In addition, primers for some deer-specific species were used that had been developed separately by Bisset *et al.* (unpublished). These included *Spiculopteragia spiculoptera* and *Spiculopteragia asymmetrica, Trichostrongylus askivali* and *Oesophagostomum sikae*. Consecutively, single or multiplex PCR tests were optimized.

For the development of the deer GIN primers by Bisset *et al.* (unpublished), samples of adult males were obtained from the Massey University deer farm, Palmerston North. Adult males identified to species were lysed in a 3% solution of proteinase K (Roche, Basel, Switzerland) in Direct PCR lysis reagent (Viagen Biotech, Los Angeles, USA), and then the ITS-2 rDNA regions were amplified, cloned and sequenced following the methodology described by Bisset *et al.* (2014).

Table 4.1 PCRs primers used to identify GIN infective larvae in deer in New Zealand.

Generic primers		
Pattern	Primer name	Sequence (5'-3')
Forward	ITS2GFnest	CACGAATTGCAGACGCTTAG
Reverse	ITS2GRnest	GCTAAATGATATGCTTAAGTTCAGC

Species specific primers				
Organ	Host spp.	Nematode spp.	Primer name	Sequence (5'-3')
Abomasum	cattle/ sheep	*T. axei*	Trax Fd2	GATGTTAATGTTGAACGACATTAATATC
	deer	*O. leptospicularis*	Oele Fd2	CATGCAACATAACGTTAACATAATG
		S. asymmetrica	Spas Fd1	GAATAACATATGCAACATAACGTTGT
		S. spiculoptera	Spspi Rv1	GATACATGAACAATGATTGTCATACAA
		T. askivali	Trask Fd1	GTTTGTCGAATGGTCATTGTCGTAC
	sheep	*H. contortus*	Haco Fd3	CATGTATAGCGACGATGTTCTT
		T. circumcincta	Teci Fd3	AAACTACTACAGTGTGGCTAACATA
			Teci Rv1	GTACATTCAAATAGTAGCAATACGC
Small Intestine	cattle	*C. oncophora*	Coon Rv1	CTATAACGGGATTTGTCAAAACAGA
	sheep	*C. curticei*	Cocu Rv1	TGGGATTTGTCAGAACCAATGTA
			Cocu Rv2	TGAGTACACTTAAACAGTGATAATAGA
		N. spathiger	Nesp Rv1	CATTCAGGAGCTTTGACACTAAT
		T. colubriformis	Trco Rv1	ACATCATACAGGAACATTAATGTCA
		T. vitrinus	Trvi Fd1	ATGTGAACGTGTTGTCACTGTTTA
Large Intestine	deer	*O. sikae*	Oesik Rv1	TCACAGTGACAATGAGATCACG
	sheep	*C. ovina*	Chov Fd2	CAGCGACTAAGAATGCTTTGG
		O. venulosum	Oeve Rv1	CGACTACGGTTGTCTCATTTCA
			Oeve Rv2	ATACATGCATGCATACATCACATG

The larvae DNA extraction was largely as described by Bisset *et al.* (2014) and is detailed in Appendix 7, SOP 8. In brief, individual larvae were randomly selected and placed in individual 200 µl thin-walled tubes. Larvae were lysed by adding a mixture of 3% solution of proteinase K (Roche, Basel, Switzerland) in Direct PCR lysis reagent (Viagen Biotech, Los Angeles, USA). To extract the DNA, the mixture was then subject to incubation 55^0C for 16 h; 90^0C for 1 h; 4^0C for 1 min in a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany). These lysates were diluted 1:2 and used directly as a template for the PCR reaction.

The PCR reaction for the identification of larvae includes reverse or forward primers for each species, together with two conserved "generic" primers (reverse and forward) as shown in Table 4.1. Consequently, when the species is present, two products should be amplified being the product of the generic primer to ensure that DNA was present regardless of the species and the product of the species primer whose size shows the species that is present. The full protocol for undertaking the PCR reactions is in Appendix 7, SOP 8.

A series of PCR reactions were undertaken to identify the larvae (Table 4.2). The first include primers for the species considered to be most common in deer, together with some less common species which had primer sizes that fitted within the multiplex. Later reactions included other species. All the larvae were tested with the first reaction, with those not identified subsequently included in successive reactions, until they were identified.

Due to the number of reactions involved, a shortage of DNA for later reactions prevented the identification of some larvae and for some there was no PCR product regardless of the number of reactions, so their identification was not possible.

Some issues with cross-reactions of primers were observed and these were resolved by retesting some larvae in a specific order as detailed below. A specificity assay was run to confirm these were suitable by testing DNA recovered from adult male nematodes:

1. The *T. colubriformis* primer Trco Rv1 also selected *Oesophagostomum sikae* and *Trichostrongylus askivali* whereas *O. sikae* and *T. askivali* primers were specific for these species. Consequently, these latter two primers were used in reactions before the *T. colubriformis* primer was used.

2. The *Trichostrongylus vitrinus* primer Trvi Fd1 cross-reacted with *S. asymmetrica* and *S. spiculoptera*. In contrast the *S. spiculoptera* and *S. asymmetrica* primers were specific for their respective species and did not cross-react with *T. vitrinus*. Even though *S. asymmetrica* was included in Reaction 1 and 2 and *S. spiculoptera* in Reaction 1, there were a number of samples positive to *T. vitrinus* in Reaction 4. Consequently, all of the DNA samples positive for *T. vitrinus* were then retested with both Reaction 7.1 using the *S. asymmetrica* primer (Spas Fd1) and with Reaction 7.2 using the *S. spiculoptera* primer (Spspi RV1) as individual reactions. As a result, many of these were identified as *S. spiculoptera* and only a few as *S. asymmetrica* leaving 31 identified as *T. vitrinus*.
3. The primer Teci Fd3 used in Reaction 4 for the identification of *T. circumcincta* also reacted to *O. leptospicularis.* Therefore, all the positives to *T. circumcincta* using this primer were rechecked in Reaction 6 with another *T. circumcincta* primer (Teci Rv1) and with an *O. leptospicularis* primer (Oele Fd2).

Table 4.2 Reactions order and primers used to identify the larvae.

Reaction order	Primers	Species
1	Spas Fd1	*S. asymmetrica*
	Oeve RV2	*O. venulosum*
	Coon RV1	*C. oncophora*
2	Trask Fd1	*T. askivali*
3	CocuRV1	*C. curticei*
	Spas Fd1	*S. asymmetrica*
	Spspi RV1	*S. spiculoptera*
	Oesik RV1	*O. sikae*
4	Trax Fd2	*T. axei*
	Trvi Fd1	*T. vitrinus*
	Teci Fd3	*T. circumcincta*
	Trco Rv1	*T. colubriformis*
	Chov Fd2	*C. ovina*
5	Cocu Rv2	*C. curticei*
	Nesp Rv1	*N. spathiger*
	Coon Rv1	*C. oncophora*
	Haco Fd3	*H. contortus*
	Oeve Rv1	*O. venulosum*
6 [1]	Oele Fd2	*O. leptospicularis*
	Teci Rv1	*T. circumcincta*
7.1 [2]	Spas Fd1	*S. asymmetrica*
7.2 [3]	Spspi RV1	*S. spiculoptera*

[1]Reaction run on positives to *T. circumcincta* in Reaction 4 as well as larvae not identified in previous reactions

[2] Reaction run on all positives to *T. vitrinus* in Reaction 4 as well as larvae not identified in previous reactions

[3] Reaction run on all positives to *T. vitrinus* in Reaction 4 as well as larvae not identified in previous reactions

4.2.3 *Statistical analysis*

Statistical analyses were carried out using SAS (Statistical Analysis System, version 9.3; SAS Institute Inc., Cary, NC, USA). Pairwise comparisons of the prevalence between all species were performed using a logistic regression model using the LOGIT procedure. The level of significance to declare significant differences between the prevalance of two species was adjusted using the Bonferroni adjustment according to the total number of pairwise comparisons. Effects of island (North/South), season (mild/cold) and farm type (D, DS, DC, DSC) on the prevalence of each species were evaluated with one-way analysis of variance for each of the factors using the GLIMMIX procedure. Two-way analysis of variance was not possible because there were not enough observations for each of the combinations between levels of two factors.

4.3 Results

4.3.1 *Prevalence by species and farm type.*

The prevalence of each species (Table 4.3) calculated as a percentage of the 1217 positive larvae samples calculated as: the prevalence overall; prevalence by island; host species present on farms; and season.

Table 4.3 Total, island, host species on farms, and season prevalence (%) of each nematode species, in deer 12-24 month of age.

Larvae species	Total larvae (n)		Island		Host species				Season	
			South	North	D	DS	DC	DSC	Mild	Cold
O. venulosum	45[o]	(548)	40[a]	38[a]	37[b]	53[a]	43[ab]	29[c]	28[b]	54[a]
Ostertagia-type[1]	34	(411)	31[a]	27[a]	38[a]	22[b]	22[b]	29[b]	36[a]	20[b]
S. asymmetrica	14[p]	(173)	12[a]	13[a]	13[a]	10[a]	12[a]	12[a]	15[a]	10[b]
S. spiculoptera	10[pq]	(124)	8[a]	9[a]	13[a]	8[ab]	4[b]	6[b]	14[a]	3[b]
O. leptospicularis	9[q]	(114)	10[a]	5[b]	10[a]	3[b]	6[ab]	9[a]	7[a]	6[a]
C. oncophora	4[r]	(52)	1[b]	7[a]	1[b]	4[ab]	2[b]	6[a]	4[a]	2[a]
T. askivali	3[rs]	(40)	3[a]	2[a]	2[a]	3[a]	2[a]	3[a]	2[a]	3[a]
T. axei	3[rs]	(35)	1[p]	4[a]	1[b]	1[b]	8[a]	1[b]	1[a]	3[b]
H. contortus	3[rs]	(33)	3[a]	1[b]				[2]		
T. vitrines	3[rs]	(31)	2[a]	3[a]				[2]		
T. circumcincta	2[rs]	(27)	3[a]	1[b]				[2]		
O. sikae	2[st]	(20)	2[a]	1[b]				[2]		
T. colubriformis	1[st]	(18)	2[a]	0[b]				[2]		
C. curticei	0[t]	(2)	0[a]	0[a]				[2]		
total identified	100	(1217)								
total larvae		1416	816	600	384	168	240	624	624	792
unknown /no DNA		199								

[1] Includes the deer-specific species *S. asymmetrica, S. spiculoptera* and *O. leptospicularis.*
[2] The analysis was not performed because of low prevalence.
[a, b,c] Least squares means of the prevalence with different superscript letters in the same row and effect (island; host species; season), are significantly different ($p < 0.05$).
[o,p,q,r,s,t] Least squares means of the total prevalence with different superscript letters in the same column, are significantly different ($p < 0.05$). Note: where differences have a lower p-value, they are given in the text.
Mild =includes summer, spring and autumn. Cold= winter.
D=Deer only farm. DS= deer and sheep farm. DC= deer and cattle farm, DSC= deer, sheep and cattle farm.

O. venulosum was the most prevalent species. Even when all the deer *Ostertagia*-type nematodes were combined in one group and compared to *O. venulosum* this was significantly different ($p < 0.0001$) between them. The *Ostertagia*-type nematodes as a group were significantly more common than the abomasal *Trichostrongylus* spp. (6%) considered as one group ($p < 0.0001$).

For *O. venulosum*, the prevalence was significantly higher on DS farms compared to D and DSC ($p<0.001$) and they were more common in the Cold Season than in Mild Season ($p<0.0001$).

The second most common species identified were the deer *Ostertagia*-type spp., of which *S. asymmetrica* was the most prevalent and was significantly more common than *O. leptospicularis* ($p=0.02$). The deer *Ostertagia*-type nematodes, as one composite group, had a higher prevalence on D farms and this difference was significant ($p<0.01$) compared to DS, DC and DSC There were also more *Ostertagia*-type species during the Mild Seasons compared to the Cold Season ($p<0.0001$).

Within the group of *Ostertagia*-type species there are some variations. For *S. asymmetrica*, there was no significant difference between farm types. For *S. spiculoptera* a higher prevalence was seen in the D farms compared to the DC and DSC farm types ($p<0.001$) but this was not significantly different from DS ($p>0.05$). For *O. leptospicularis* a higher prevalence was seen in the D farms than DS farms ($p=0.01$) but this was not significantly different to DC or DSC farms ($p>0.05$).

For other species there was generally a low prevalence identified.

4.3.2 Prevalence by farm

The prevalence was calculated by farm with a farm being considered positive when one or more of the 24 larvae per farm was identified as one of the species. These are shown in Table 4.4.

Table 4.4 Farm level (n=59) prevalence of each nematode species in deer

Prevalence of species by farm		
Larvae species	Positive farms	
	%	(n)
Ostertagia-type[1]	88	(52)
O. venulosum	83	(49)
S. asymmetrica	73	(43)
O. leptospicularis	47	(28)
S. spiculoptera	47	(28)
T. askivali	32	(19)
C. oncophora	29	(17)
T. circumcincta	29	(17)
T. vitrinus	29	(17)
T. axei	19	(11)
T. colubriformis	19	(11)
O. sikae	17	(10)
H. contortus	12	(7)
C. curticei	3	(2)

[1] Includes the deer-specific species *S. asymmetrica, S. spiculoptera* and *O. leptospicularis* which are also shown separately below.

At farm level, *O. venulosum* was the most prevalent single species. The second most prevalent was *S. asymmetrica*, followed by *O. leptospicularis* and *S. spiculoptera*. However, the deer *Ostertagia*-type nematodes as a composite group had a higher prevalence by farm than *O. venulosum*. Of the species identified, the least prevalent nematode was *C. curticei* only found on 2 farms.

4.4 Discussion

This study has identified the most common gastrointestinal nematode species present in deer and on deer farms across a range of farm types within New Zealand. The approach taken was to opportunistically utilise the faeces collected from a large cross-section of farms taken for a study of Johne's disease and to culture the remaining faecal material to then recover 24 L3 per farm to identify and describe the prevalence of different species using molecular tools.

At this time there is no formally described set of primers that can be used in a PCR for this purpose for all the species that might infect red deer. Consequently, a mixture of those described by Bisset *et al.* (2014) for common species in sheep, and to a limited extent cattle, were combined with others developed as required (Bisset unpublished; this study) to provide coverage of a total of 15 species that have been found in deer. It was a challenge to work around the cross-reactions that were identified when using PCR reactions for some deer species with those only found in sheep and cattle, but this was resolved by developing new primers, re-testing some larvae and running the reactions in a specific order.

The distribution of selected farms was not even over the country, largely because deer farms are only common in some areas so it was only possible to analyse prevalence by geographical distribution at the level of the North or South Islands. In the analysis of the prevalence by season, it was only possible to examine winter (Cold) versus all other seasons combined (Mild). This was only undertaken for those species with a sufficiently high prevalence for the analysis to be robust. The interaction of host species with season was not included in the analysis because in the Mild season there was data for only one "DS" farm.

The present study utilised deer that were greater than one year of age. These animals should have a reasonably well developed immune response to GIN and this may have influenced the composition of their burdens (Sykes 1994). A different prevalence may have been noted if younger animals were sampled. A further factor which could influence the results is the fecundity of different species meaning the prevalence does not necessarily reflect worm burdens in animals. Larger nematodes such as *Oesophagostomum* are generally more fecund than small trichostrongylid species. Most species will have a reduced fecundity in the presence of a developed immune response.

This is particularly obvious with *T. circumcincta* in sheep (Stear *et al.* 1997) and so by extrapolation is very likely to also occur with *Ostertagia*-type species in deer. Regardless of these limitations, the results still provide useful and robust information about the presence of different GIN species in deer in New Zealand.

For those larvae identified, *O. venulosum* was the most prevalent species at farm level and in overall frequency. In reported studies with red deer in New Zealand this species is consistently present although with different frequencies (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b; Hoskin *et al.* 2005). In naturally infected 4-month-old deer, Hoskin *et al.* (2005) reported a higher burden of *O. venulosum* (n=772) compared to *Ostertagia*-type (n=273) nematodes. It is known that the strongylid nematode *O. venulosum* is highly fecund (Koprivnikar and Randhawa 2013) and the results found in this study do not consider the relationship between number of worms present and the egg count so this will potentially bias the interpretation of the prevalence figures.

Oesophagostomum venulosum is considered to be a normal parasite of sheep and it is not considered to be very pathogenic because unlike other members of this genus, the larval stages do not induce the formation of inflammatory nodules when animals are parasitised under field conditions. Even though its normal host is considered to be sheep, the infectivity in deer appears high (McKenna 1997; Hoskin *et al.* 2000b; McKenna 2009a). In the present study it was higher in farms that also grazed sheep compared with deer-only farms implying cross-infection may be common. In a related cross-infection study (Tapia-Escárate *et al.* 2015b; Chapter 5) utilizing sheep-origin infective larvae, the establishment rate of *O. venulosum* was significantly lower in red deer than in sheep but 5.8 % of the infective dose still established compared with 21.6 % in sheep.

In Australia *O. venulosum* has been described as more common in winter-rainfall areas (Cole *et al.* 1986) but under New Zealand conditions it would be considered a "normal" deer parasite observed equally commonly in both islands. In lactating ewes it was observed to be similarly common across a range of farm types and locations (Hervé *et al.* 2003). Whilst other species from this genus, including *O. radiatum*, and *O. columbianum* struggle in areas with cold winters (Sutherland and Scott 2009), the free-living stages of *O. venulosum* are able to survive on pasture over winter in temperate areas (Taylor *et al.* 2007) so it is not surprising it is reasonably common in New

Zealand. A notable result was the higher prevalence of *O. venulosum* in winter. However, as most of the samples from DS, where the highest prevalence of this nematode was found, were taken in winter (144 of 168 larvae) the results may have a bias due to the time of collection of samples.

The next most common larvae identified were the *Ostertagia*-type species of deer. Of these *S. asymmetrica* was the most prevalent followed by *S. spiculoptera* and then *O. leptospicularis.* This high prevalence of the *Ostertagia*-type spp. is consistent with the high frequency found in other studies in red deer in New Zealand (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b; Hoskin *et al.* 2005).

The relationship between egg count and worm burden can be particularly poor with nematodes in the Ostertagiinae subfamily. In studies with red deer in New Zealand a low correlation between egg counts and nematode counts of *Ostertagia*-type nematodes during spring and summer has been reported with some animals having zero faecal egg counts with as many as 10,000 *Ostertagia*-type nematodes (Mackintosh *et al.* 2014a). Consequently, the approach taken for this survey may underestimate the true prevalence of these species of nematodes. Nevertheless, these *Ostertagia*-type nematodes, as one group, had a higher prevalence on the exclusively deer farms and this difference was significant compared to DS, DC and DSC. This is also consistent with the results from the cross-grazing study (Chapter 6) where *Ostertagia*-type burdens were lower in deer when cross-grazing with sheep or cattle.

When the results are considered by nematode species, there are some differences. There was no difference in *S. asymmetrica* prevalence between farm type but *S. spiculoptera* was more common on D farms compared with DC and DSC. Whilst these two species are considered to be host-specific for deer, the third species *O. leptospicularis* is also known to readily parasitize cattle (Bisset 1980). Consistent with this is the observation that *O. leptospicularis* had a higher prevalence on D farms than DS but there was a similar prevalence on D, DC and DSC farms. By contrast, in the cross-grazing study (Chapter 6), *O. leptospicularis* was the most prevalent *Ostertagia*-type species in all the treatment groups but there were differences between some groups. In that cross-grazing study the deer grazing on their own had the highest numbers of this nematode species being almost significantly higher than the deer cross-grazing with cattle and this was significantly higher than those grazing with sheep. Also in that study it was notable there were high burdens of *O. leptospicularis* on one occasion when deer were cross-grazing with cattle on the Invermay farm.

T. askivali was identified in 2009 in New Zealand (McKenna 2009c) in deer from the Massey University Deer Unit. It may have been previously misidentified as *T. axei* for many years since these two species are morphologically very similar and both are located in the abomasum. The presence of *T. askivali* on 32% of the deer properties indicates it is common and that it was most likely previously misidentified. The recent discovery of this species in New Zealand means we know little about it in terms of anthelmintic efficacy and pathogenicity. This species was originally described from red deer in Scotland (Dunn 1965), the most common origin of red deer in New Zealand.

T. axei is a species which is capable of infecting a variety of hosts including deer with little host preference (Borgsteede 1981). In this study the prevalence of this nematode species was relatively low (3%), but the prevalence in "DC" farms was significantly higher at 8% implying that the presence of cattle contamination of pasture was increasing the prevalence of *T. axei* in deer. Similarly, a study in Australia found higher numbers of *T. axei* in sheep when they were co-grazing with cattle (Arundel and Hamilton 1975). By contrast, in the cross-grazing study (Chapter 6), deer with the highest burden of *T. axei* were observed in the group cross-grazing with sheep. In cross-infection studies of sheep or cattle parasites into red deer it was found that the infectivity of *T. axei* was higher for sheep than red deer (Tapia-Escárate *et al.* 2015b;Chapter 5) but the same as for cattle (Tendoesschate *et al.* unpublished;Tapia-Escárate *et al.* 2015a). According to Ross and Purcell (1969) different strains of *T. axei* have a predilection for different host species, and this may be the reason for differences in these studies.

O. sikae nematodes were originally identified in young housed deer that were artificially infected with larvae from naturally infected deer at Massey University (Chapter 2). In that study, large numbers of *Oesophagostomum* spp. were recovered and extensive pathological damage in the large intestine was found in the infected animals, to the extent that the study was terminated early. The findings in this present study indicate their presence is not unusual, since it was found on 17% of the properties in both the North and South Islands. Morphologically these parasites resemble *Oesophagostomum radiatum,* usually found in the large intestine of cattle. McKenna (1999) reported finding *O. radiatum* in adult deer but it is possible they were *O. sikae* that were misidentified at that time. At the molecular level these parasites can be clearly distinguished from *O. radiatum* (Chapter 2). In this study there was no attempt to identify *O. radiatum* so it remains uncertain if they were present or not. *O. radiatum* in cattle is relatively uncommon in New Zealand and is acknowledged to be a species that is more important in tropical and subtropical areas (Taylor *et al.* 2007).

The presence of *Cooperia oncophora* on 17 properties implies that this species can cross-infect between cattle, its normal host, and deer, although it can also be found in sheep. The prevalence found for *C. oncophora* in this trial contrasts with the cross-grazing study (Chapter 6), where this species was not found. It also was not found when cattle nematodes were used to infect young red deer in a cross-infectivity study (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a). As a relatively uncommon species in this survey the statistical differences between *C. oncophora* and others need to be interpreted with caution. Although a higher prevalence was seen in the DSC farms than DC and D, it was not different to DS. Although the total prevalence was low, on one DSC farm all 24 larvae were *C. oncophora,* so the results must be interpreted with caution. Similarly, care should be taken not to assume their worm burdens were particularly large. Such features need to be further studied before robust conclusions can be considered. The related *Cooperia* species in sheep (*C. curticei*) was only found on one property and then it was only one larva, indicating this sheep species is unlikely to be an issue for deer. *C. oncophora* is considered to be only a mildly pathogenic species in cattle. It could be hypothesised it may be the same for deer but further data is needed before final determinations can be made. Of more importance is that *C. oncophora* is a dose-limiting species for some anthelmintics including the macrocyclic lactones (MLs) and the prevalence of ML-resistant *C. oncophora* in cattle is high. Therefore, if they are cross or co-grazing with cattle, resistance may be the case in deer as well.

Haemonchus contortus has been found in a range of ruminants including deer although its preferred hosts are sheep and goats. In cross-infection studies of the ability of sheep or cattle parasites to infect red deer, it was found that the establishment rate of *H. contortus* was higher for sheep (19%) than red deer (11%) (Tapia-Escárate *et al.* 2015b; Chapter 5), but the difference was not significantly different between deer (19%) and cattle (8%) (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a). Given these establishment rates, sufficient burdens could build up in deer to be clinically significant if the environmental conditions are favourable. The low prevalence found in this study concurs with other studies in deer in New Zealand where this nematode has been reported with low burdens (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b). The prevalence of this nematode in sheep in New Zealand was recorded as 22% in the North Island and 2% in the South Island (Hervé *et al.* 2003). It is known that this nematode prefers warm tropical and subtropical areas because larval development requires relatively high temperatures and humidity. In contrast to the prevalence in sheep, in this study the prevalence was greater in the South Island, although it has to take into account that most of the farms sampled in the South Island were in the Canterbury region where *Haemonchus* may occur and 97% of the larvae of *H. contortus* found were from deer sampled during the mild season (spring/autumn).

Overall this project used PCRs to identify the prevalence of a wide range of species of nematodes in deer. The protocol allowed the identification of the most likely common species in deer with relatively high efficiency. This project highlights that further work on developing and validating better primers without cross-reaction between species found in deer needs to be undertaken before this approach can be more widely adopted.

Chapter 5 Establishment rate of sheep gastrointestinal nematodes in farmed red deer (*Cervus elaphus*).

Sections of this chapter have been published:

Tapia-Escárate, D., Pomroy, W., Scott, I., Wilson, P., Lopez-Villalobos, N., 2015b, Establishment rate of sheep gastrointestinal nematodes in farmed red deer (Cervus elaphus). Veterinary. Parasitology. 209, 138-14.

(Appendix 8)

5.1 Introduction

As in other livestock production systems, parasites are an important clinical and economic problem in farmed deer (Audigé *et al.* 1998; Wilson 2002). Whilst most focus has historically been on clinical disease caused by *Dictyocaulus* spp., gastrointestinal nematodes (GIN) may also be an issue for red deer (Mason 1977; Watson and Charleston 1985a; Audigé *et al.* 1998). To help limit parasitism in deer there has been a move by deer farmers to use integrated management systems, particularly cross-grazing with other ruminants, to restrict the number of deer-specific parasite larvae on pasture. However, very few studies have investigated the potential for cross-infection of GIN between deer and other ruminants. It is known that deer can be infected with some GIN of sheep including *Trichostrongylus axei, Haemonchus contortus, Oesophagostomum venulosum, Teladorsagia circumcincta, Trichostrongylus vitrinus, Nematodirus* spp., *Chabertia ovina* (McKenna 2009b). Nevertheless, it is not clear how readily deer are infected with sheep nematodes. The aim of the present study was to determine the establishment rate of sheep GIN in young deer compared with sheep of the same age to help understand the potential risks associated with cross-grazing and susceptibility of deer to sheep GIN.

5.2 Material and Methods

Five male red deer calves (*Cervus elaphus*) and five Romney-cross ewe lambs (*Ovis aries*) both raised on pasture were housed in different sheds. Animals of both species were born mid-November to early December 2011 and thus were aged 5-6 months. The deer were treated with abamectin (0.2 mg/kg; Combat AbaCare LV®, Virbac New Zealand Ltd) together with oxfendazole (9.06mg/kg; Bomatak C®, Bayer New Zealand Ltd), and lambs were treated with abamectin (0.2 mg/kg, Combat AbaCare LV ®) together with a dual combination of oxfendazole (4.53mg/kg) plus levamisole HCl (8mg/kg; Scanda®, MSD Animal Health, NZ Ltd), and monepantel (2.5mg/kg; Zolvix®, Novartis New Zealand Ltd). Two weeks after treatment they were infected with a mixed culture of sheep-origin GIN including *Trichostrongylus colubriformis, T. vitrinus, T. axei, T. circumcincta, H. contortus, Cooperia curticei, Nematodirus* spp., *O. venulosum* and *Chabertia ovina*. Each animal was given 327 infective larvae (L3)/kg liveweight administered by stomach tube (Table 5.1).

Larvae were available as individual experimental isolates for *T. colubriformis* and *T. circumcincta* with the remainder collected from naturally infected sheep. The infective dose given to deer was within the range nominated for sheep in WAAVP guidelines for evaluating anthelmintic efficacy in ruminants (Wood *et al.* 1995). Larvae were less than five month of age at the time of infection. Four weeks after infection all animals were euthanized. After slaughter the abomasa, small intestines and large intestines were removed and frozen at -20°*C* until processing for worm counts. Individual organs were thawed, and then worm counts were undertaken on 5% aliquots of abomasal and small intestinal washings, and 10% aliquots of large intestinal washings. Prior to counting, all aliquots were sieved over a 37.5 μm mesh. All available males up to a maximum of 50 per animal, for each genus, were examined and identified to species. These proportions were used to calculate the worm burdens of each species within a genus for each animal.

When the species in the original dose were known, the establishment rate was estimated by comparing the worm burden with the infective dose. These establishment rates were compared after ArcSin transformation and analyzed with a linear model that included the fixed effect animal species (sheep vs deer) and a random residual error. To enable a comparison between sheep and deer for *Trichostrongylus axei* and *Oesophagostomum venulosum* the number of worms counted was divided by the total number of infective larvae given of *Trichostrongylus* spp. and the *Oesophagostomum/Chabertia* group and this proportion was then compared as for the other species. To provide an estimate of establishment rate for these two species, the number of worms of each species counted in the sheep was extrapolated to estimate the proportion in the infective dose and this was used to estimate the establishment rates for those species. The experiment was approved by the Massey University Animal Ethics Committee.

5.3 Results

On the day of challenge all animals had zero faecal egg counts and on Day 28 all animals shed eggs with the mean for sheep of 4750 eggs/g (range 1200-4950) and for deer 350 eggs/g (range 50-800). No clinical signs of parasitism were seen in any animal. Individual and mean establishment rates for both deer and sheep are shown in Table 5.1. For Sheep 418 the small intestinal counts from the first aliquot indicated an unusually high establishment rate of *Trichostrongylus,* so a reserve 5% aliquot was also counted and the worm count for the small intestine of this animal is based on both aliquots. Overall the establishment rates were low in deer compared with the sheep. The arithmetic mean establishment rate in deer for *H. contortus* was significantly lower than in the sheep (10.7 vs. 18.8%; p=0.0118). Almost no *T. circumcincta* established in deer compared with a mean of 35.8% in sheep (p<0.0001). Similarly, only two *C. curticei* were found in the 5% aliquot of one deer whereas a mean of 31.1% established in sheep (p<0.001). No other species of *Cooperia* were seen. No *Nematodirus* were seen in any animal. The arithmetic mean proportion of *Trichostrongylus* which established in sheep was 73.9% compared with 1.0% in deer (p<0.0001). There were no *T. colubriformis* or *T. vitrinus* found in any deer but both species were found in all sheep. The mean *T. axei* count in sheep was 64 (range 20-120) and in deer was 30 (range 20-50). For sheep the mean proportion of *T. axei* compared with other *Trichostrongylus* species was 8.6%. If the ratio of *Trichostrongylus* species found in sheep is assumed to reflect the proportion in the challenge dose, it was estimated that the establishment rate of *T. axei* in deer was 12.2% compared with 66.7% in sheep which was different between the two hosts (p<0.01). There were no *C. ovina* found in any deer, only *O. venulosum,* whereas in sheep *C. ovina* was seen in four of the five. The larvae of *Oesophagostomum* and *Chabertia* were counted together in the infective dose. The mean combined percentage of *Oesophagostomum* and *Chabertia* establishing in sheep was 21.8% compared with 5.3% in deer (p=0.0002). In sheep *Oesophagostomum* comprised a mean of 91.9% of large intestinal parasites. If this ratio is extrapolated to reflect the proportion in the challenge dose, then the estimated establishment rate of *O. venulosum* in sheep was 21.6% compared with 5.8% in deer, which was different between the two hosts (p<0.05). In sheep the mean burden of *O. venulosum* was 472 (range 80-860) and in deer was 336 (range 40-490).

Table 5.1 Mean establishment rate of mixed challenge of nematodes to young sheep (n=5) and deer (n=5).

		H.contortus	*T.circumcincta*	*C.curticei*	*Trichostrongylus* spp.	*Oesophagostomum* + *Chabertia* spp.
Animal	LW (kg)	Establishment rate (%)[1] Deer				
117	64	13	2	0	2	7
118	60	9	1	0	1	1
120	60	6	0	0	1	6
122	58	12	1	0	1	8
123	63	13	3	2	1	5
Arithmetic Mean	61	10.7	1.4	0.4	1	5.3
LS Mean [2] (SE)		10.51 (0.025)	1.02 (0.041)	0.07 (0.039)	1.01 (0.049)	4.81 (0.064)
		Establishment rate (%)[1] Sheep				
409	21	18	28	27	63	23
416	27	11	24	27	58	23
417	23	23	51	43	78	37
418	34	21	34	15†	90†	2
420	19	20	41	39	80	24
Arithmetic Mean	24.8	18.8	35.8	31.1	73.9	21.8
LS Mean [2] (SE) [3]		18.6 (0.025)	35.53 (0.041)	30.72 (0.039)	74.85 (0.049)	19.94 (0.064)
P[4]		0.0118	<0.0001	<0.0001	<0.0001	0.0284

† based on 10% aliquot for the small intestine.

[1] Number of worms counted/number of infective larvae administered.

[2] Least square means.

[3] Standard errors
[4] Probability of significant differences between deer and sheep.

5.4 Discussion

This is the first report of the establishment rate of sheep-origin GIN in farmed red deer. The establishment rate in deer of the sheep GIN used in this study was generally low relative to that in sheep, with the highest rate in deer being for *H. contortus, T axei* and *O. venulosum*. Sheep and deer that were used in this study were of a similar age of 5-6 months, but still both hosts had enough time to develop some acquired immunity and how this could have affected the final worm burdens could not be assessed.

A number of reports have noted the occurrence of sheep GIN in deer including the consolidated checklist of helminth parasites of terrestrial mammals in New Zealand (McKenna 2009b). However, those reports do not give any indication as to the establishment rate of these species, which is a critical question for understanding the potential effectiveness of cross-grazing sheep:deer for parasite control. In Italy a survey of wild deer found a prevalence of 1 and 1.3% for *T. circumcincta* and *H. contortus,* respectively, but the numbers of worms recovered were low for both species (Manfredi *et al.* 2007). In studies in New Zealand both those species have been found in housed deer infected with parasites from naturally infected deer but burdens were also low (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b). By comparison, in the present study the relatively high establishment rate of *H. contortus* and *T. axei* in deer compared with most other nematode species does suggest the possibility that pathogenic burdens could develop if deer were grazing pasture contaminated with these worm species.

The only *Cooperia* species found in this study was *C. curticei* and the results indicate it does not easily establish in red deer, since only 2 worms were seen in the 5% aliquot in one animal. Other species of *Cooperia* including *C. pectinata, C. oncophora* and *C. punctata* have been found in red deer in New Zealand and elsewhere (McKenna 2009b). The latter two species have been reported in sheep but did not appear in the present study.

The relatively high establishment of *Trichostrongylus* spp. in sheep is consistent with that reported for *T. colubriformis* in young sheep (Dobson *et al.* 1990). *T. axei* has been reported in deer in several studies, which is similar to other observations that this species is able to infect a range of ruminants and horses. However, burdens in naturally infected deer are generally low (Connan 1996; Santin-Duran *et al.* 2004). In New Zealand they have comprised 15% of abomasal burdens in two studies (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b) with the majority of the abomasal worms being *Ostertagia leptospicularis, Spiculopteragia asymmetrica* or *Spiculopteragia spiculoptera*. The use of morphological criteria to recognise larvae in the present study required some extrapolation to determine the establishment of *Trichostrongylus* species in particular and can only be used as an approximation. The establishment rate of *T. axei* in deer in the present study was estimated to be only 12.2% compared with 67% in sheep but the overall burden of this species was low in both hosts. The absence of any *T. colubriformis* or *T. vitrinus* indicates that neither of these species appears to favour development in the small intestine of red deer. To date *T. colubriformis* has not been recorded in red deer in New Zealand although *T. vitrinus* has (McKenna 2009b). Thus, neither of these two potentially pathogenic *Trichostrongylus* species should present a problem for grazing deer on sheep pastures, although further evidence is required to confirm this suggestion.

In the large intestine no *C. ovina* were found in the deer although this species has been recorded in deer previously (McKenna 2009b). *O. venulosum* has been recognised in grazing deer in New Zealand for several years but in two field studies the burdens were <100 nematodes (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b). In sheep *O. venulosum* have been reported to initially develop in the mucosa of the ileum, then move to the lumen of the large intestine and undergo their final moult after 16 days with a mean minimum prepatent period of 28 days (Goldberg 1951). Assuming that the rate of development is similar in red deer, any *O. venulosum* in the challenge dose should have been available to be counted if they had established. Similarly to *O. venulosum, C. ovina* larvae originally develop in the mucosa but have been reported to return to the lumen of the large intestine by 96 hours after infection. They then undergo their final moult to immature adults by 25 days, although the minimum prepatent period was reported to be 52 days (Threlkeld 1948). Thus, any *C. ovina* present should also have been available for counting if they had established, even though the worms would not yet have been producing eggs. In the present study the establishment in deer of *O. venulosum* was about a quarter of that in sheep, but this could still allow a reasonably large burden to build up in deer if grazing sheep-contaminated pasture. However, this worm species does not induce nodule formation around developing larvae as for most other *Oesophagostomum* species, and is generally considered to be of low pathogenicity (Taylor *et al.* 2007).

5.5 Conclusions

Overall, data here indicate a low establishment rate for most sheep GIN in red deer. They suggest that under field conditions *H. contortus* could build up in sufficient numbers to be a clinically significant burden. *T. axei* could also establish in sufficient numbers to create a clinical problem and as they can be a parasite of both sheep and cattle, may be an issue with deer cross-grazing with either host. Nevertheless, the establishment rate indicated here was low. *O. venulosum,* being essentially non-pathogenic, should not present a clinical problem even with modest burdens. The other three main pathogenic GIN species of sheep, being *T. circumcincta*, *T. colubriformis* and *T. vitrinus*, are unlikely to be able to build up to sufficient numbers in deer to create a clinical problem. Thus, there is potential for an organised system of cross-grazing between red deer and sheep to help reduce the challenge of GIN to deer. Future studies should concentrate on observing the effectiveness of cross-grazing with sheep under field conditions for GIN control in farmed deer. Some sheep GIN species may still exert some subclinical effects in deer if small burdens establish. Similar research is needed to determine the establishment of cattle-origin GIN in deer.

5.6 Acknowledgements

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Chapter 6 Evaluation of cross-grazing deer with sheep or cattle, as a means to control gastrointestinal and pulmonary nematodes in farmed red deer in New Zealand

6.1 Introduction

Effective parasite control is a key factor to obtain optimal growth in young deer. To date deer farming has relied on the use of anthelmintics to achieve parasite control, but since anthelmintic resistance is becoming a problem around the world in many ruminants (Prichard 1990; Kaplan 2004) including red deer in New Zealand (Mackintosh *et al.* 2014b), alternative options relying less on drugs, need to be considered to reduce the dependence on anthelmintics.

In New Zealand, deer farmers have been utilising cross/co-grazing as a management tool to manage pasture quality which is of potential benefit to reduce the exposure to gastrointestinal nematodes (GIN). The usefulness of cross-grazing for nematode control is based on the evidence that most nematodes have a preferential host and if they are ingested by another host they die or they establish at a lower rate, consequently restricting the number of infective larvae (L3) on pasture. The value of co/cross-grazing sheep and cattle for reducing pasture contamination has been understood for some time (Arundel and Hamilton 1975; Barger and Southcott 1975; Southcott and Barger 1975; Barger and Southcott 1978; Helle 1981; Inderbitzin *et al.* 1981; Donald *et al.* 1987; Jordan *et al.* 1988) but there are no studies on the usefulness of cross-grazing between deer and other ruminant animals as a means of parasite control for deer.

Red deer (*Cervus elaphus*) can be parasitized with a range of nematodes, some of which are shared with other ruminants but most are deer-specific. Those which are generally specific to deer include *Dictyocaulus eckerti*, *Trichostrongylus askivali*, *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera*, *Ostertagia leptospicularis* and *Oesophagostomum sikae*. Of these *O. leptospicularis* can successfully infect cattle (Bisset 1980; Borgsteede 1981; Bisset *et al.* 1984; McKenna 2009a) and sheep (Borgsteede 1981; McKenna 2009a). In addition, a number of nematodes whose preferred hosts are sheep and cattle have been reported in deer. These include, *Oesophagostomum venulosum, Trichostrongylus vitrinus, Haemonchus contortus, Teladorsagia circumcincta* which have a preference for sheep and *Ostertagia ostertagi, Cooperia oncophora, Cooperia punctata* and *Dictyocaulus viviparus* which have a preference for cattle (McKenna 2009a). *Trichostrongylus axei* is a species which is capable of infecting a variety of hosts with little host preference (Borgsteede 1981) and this includes deer. Even though there are many nematodes from sheep and cattle that have been described in deer, there are only some which are considered to be able to establish in deer with any reasonable success including *T. axei*, *H. contortus* and *O. venulosum* (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a; Tapia-Escárate *et al.* 2015b; Chapter 5).

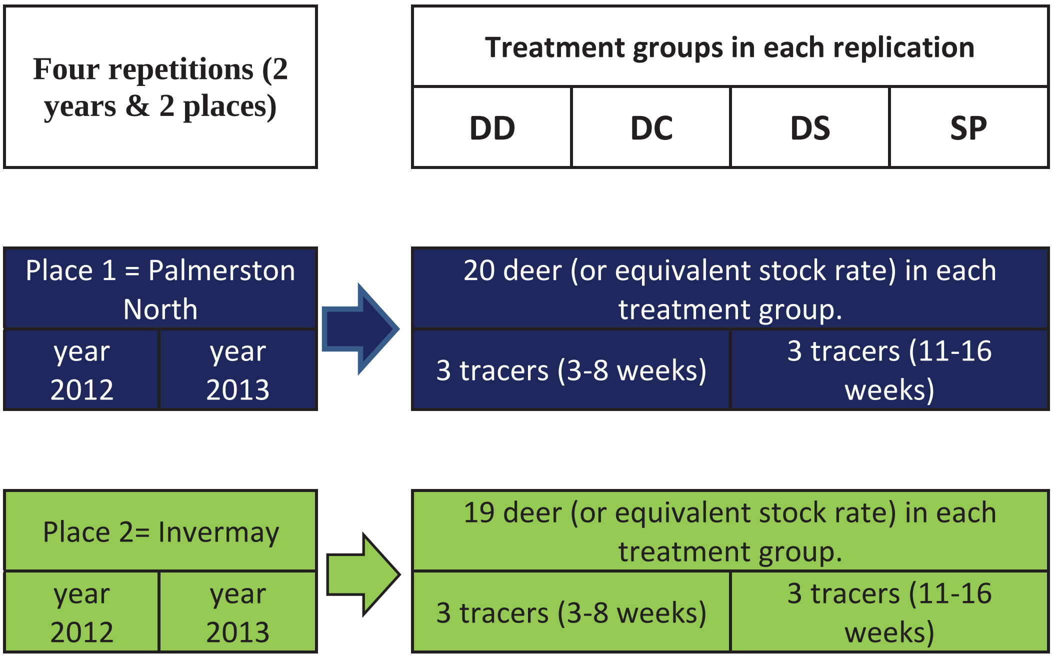
The aim of this study was to determine the value of an organised cross-grazing system between deer and sheep or cattle to assist with the control of deer internal parasites.

6.2 Materials and methods

6.2.1 Experimental design

The field study was replicated over two years (2012-2013) for 16 weeks each year at two locations: Massey University, Palmerston North (PNmassey); and Invermay Agriculture Research Centre, AgResearch, Mosgiel (InvAgR). The study in each year commenced at weaning in late February/beginning of March and concluded in June. Each replicate included four treatment groups: Deer cross-grazing with cattle (DC); deer cross-grazing with sheep (DS); deer grazing on their own (DD); and deer grazing on their own and treated with anthelmintic every two weeks to suppress the possibility of pasture infestation with GIN as a positive control (SP) (Figure 0.1). All deer were weighed every two weeks and the sheep and cattle every four weeks. Two estimates of the parasite burden and species in deer were achieved by introduction of “tracer” deer which were slaughtered after a 5-week period at weeks eight and 16.

Figure 0.1 Experimental design of cross-grazing experiment between young red deer and young sheep or cattle



DD = only deer paddocks, deer trigger treated.
DC = crossgrazing deer and cattle paddocks, deer trigger treated.
DS = crossgrazing deer and sheep paddocks, deer trigger treated.
SP = only deer suppressively treated paddocks

6.2.2 Allocation and randomization of paddocks

In both locations the area was divided into 30 paddocks of different sizes. At PNmassey they were blocked by size and then randomized to treatment each year within blocks. In InvAgR in 2012 the paddocks were blocked and these were randomly allocated such that DC and DS groups had two contiguous areas and the DD and SP groups had one area each. In InvAgR in 2013 the paddocks were randomly allocated across the whole area. In both locations, 10 paddocks were allocated for DC and DS and 5 paddocks for DD and SP. For PNmassey, 2012-2013 the areas allocated to DC and DS were ~7.6 ha and for DD and SP were ~3.8ha. For InvAgR 2012-2013 the areas allocated to DC and DS were ~4.6 ha and for DD and SP were ~2.5 ha.

6.2.3 *Preparation of paddocks prior to the starting of the trial*

All the paddocks were grazed by deer up until December of the year prior to the study. For the preparation of paddocks immediately before the study commenced the DC paddocks were grazed by adult female cattle, DS by recently weaned ewes, and DD by yearling deer. At PNmassey, SP paddocks were grazed by weaned ewes and R2 cattle and at InvAgR, by yearling deer. Paddocks were grazed at least twice during this preparation period, with the exception of PNmassey 2013 when paddocks were only grazed once from December 1, because of a drought period in that year.

6.2.4 *Animals*

There were three categories of animals:

6.2.4.1 Resident deer

In March each year, recently weaned deer were allocated as “resident” animals to each treatment (n=19 at InvAgR and n=20 at PNmassey). These were ranked by weight and then randomly allocated within sex into each of the groups, except for InvAgR in 2012 when only male deer were used. The resident deer were bred on the farm for both locations.

6.2.4.2 Tracer deer

At each location and each year, two sets of three “tracer” deer per treatment were included to graze with the resident deer for five weeks to provide an estimate of the availability of infective larvae on pasture. For PNmassey these were male weaned deer purchased commercially and for InvAgR these were bred on the farm. The first set of tracer deer were grazed with the resident deer from the commencement of the study until they were slaughtered. They were effectively treated (as determined by FEC and FLC) with an anthelmintic 5 weeks prior to slaughter. The second set immediately replaced the first set and they were also effectively treated with an anthelmintic 5 weeks prior to slaughter. The exception was at PNmassey in 2013 where the second set of tracers were grazed off the experimental paddocks due to drought conditions until they were introduced as tracers 5 weeks prior to slaughter. The first set of tracers were slaughtered in Week 8 with the exception of the SP group at PNmassey in 2012 which was mistakenly drenched in Week 6 and consequently their slaughter was delayed by three weeks (Week 11) to ensure they still had a five week period grazing pasture to be expose to parasites. The second set of tracers was slaughtered at the end of the study in Week 16. At PNmassey 2012 one tracer deer in this second set of tracers in the SP group died of yersiniosis and it was not replaced.

6.2.4.3 Sheep and cattle

Young cattle and sheep born the previous spring were purchased commercially, with the exception of sheep at InvAgR that were bred by AgResearch on an adjacent farm. The number of animals required for each treatment were estimated by calculating predicted feed consumption using the software "QGraze" (Woodward *et al.* 2001). The numbers of sheep and cattle were adjusted during the experiment to match the stock unit equivalent of deer in the experiment using this same software package.

6.2.5 Grazing Management

The original target was that deer had access to at least 1700 kg DM/ha to ensure that pasture availability was not a limiting factor in liveweight gain (LWG). Animals in all treatments were moved at the same time to the next paddock. At PNmassey animals were moved when the pasture cover for any group declined to less than 1700 kg DM/ha. At InvAgR all animals were moved on a weekly basis which generally followed this same guideline. Pasture availability was measured using a rising plate meter both before and after grazing. At PNmassey in both years due to drought conditions it was necessary to provide supplementary feed (barley/hay) when pasture covers declined to less than 1700 kg DM/ha. To circumvent this restriction the animals were rotated to new pastures three times per week. They were progressively introduced to barley from Week 7 in 2012 and in 2013 from the beginning of the trial. No supplementation was required at InvAgR. Deer and the alternating species (sheep or cattle) were separated by five paddocks in the rotation to provide the longest period possible between grazing.

6.2.6 Anthelmintic treatment

6.2.6.1 Resident deer

All deer were treated as below at the beginning of the study in each year.

The decision to subsequently treat individual resident deer in DC, DS and DD was based on any of the “trigger” criteria as follows:

1. If an individual faecal egg count (FEC) was ≥ 250 eggs/g
2. If an individual faecal *Dictyocaulus* larval count (FLC) was ≥ 100 larvae/g.
3. If an individual deer’s growth rate was less than 80% of the mean of the SP treatment group in the previous two weeks, expressed as a proportion of the individual deer’s weight compared to the proportional change in the SP group.

Anthelmintic treatment was given on the day of sampling/recording if the trigger reached was growth rate, or 1-2 weeks later if the trigger was FLC or FEC as it took time to process the faecal samples.

The anthelmintic treatment used for all resident deer was oral abamectin (0.2 mg/kg; Combat AbaCare LV®, Virbac New Zealand Ltd) together with oral oxfendazole (4.53 mg/kg; Bomatak C®, Bayer New Zealand Ltd).

The SP groups were regarded as minimally parasitized due to repeated anthelmintic treatments and therefore assumed to have optimal growth rate within the constraints of the study and against which values from DC, DS and DD were compared.

6.2.6.2 Tracer deer

Tracer deer were effectively treated (as determined by FEC and FLC) with short-acting anthelmintics when they entered the study and five weeks prior to slaughter, except the first set of tracer deer in InvAgR year 2012, which were euthanized four weeks after the treatment. At PNmassey the treatment comprised Combat AbaCare LV® together with Bomatak C®. At InvAgR the treatment comprised a double dose of oral oxfendazole (9 mg/kg; “Oxfen C”, Ancare NZ Ltd) prior to slaughter.

6.2.6.3 Cattle and sheep

Cattle and sheep were given anthelmintic treatment at the beginning of the field trial and every four weeks during the study.

At PNmassey the treatment for sheep in 2012 comprised monepantel (2.5 mg/kg; Zolvix®, Novartis New Zealand Ltd) but this was changed for the last two treatments to monepantel together with oxfendazole+levamisole (4.5 +8 mg/kg: Scanda®, Schering Plough Animal Health Ltd). In 2013 the treatment was derquantel+abamectin (2 +0.2 mg/kg: Startect®, Pfizer NZ Ltd).

At PNmassey the treatment for cattle in 2012 comprised oral abamectin (0.2 mg/kg; Combat AbaCare LV®, Virbac New Zealand Ltd) together with oral oxfendazole (9.06 mg/kg; Bomatak C®, Bayer New Zealand Ltd) but this changed for the last two treatments by replacing oxfendazole with oxfendazole+levamisole (4.5mg/kg oxfendazole + 8 mg/kg levamisole: Scanda®, Schering Plough Animal Health Ltd). In 2013 the treatment was oral abamectin (Combat AbaCare LV® with oxfendazole+levamisole (Scanda®) as at the end of 2012.

At InvAgR the treatment for cattle and sheep comprised 0.2 mg/kg abamectin and 8 mg/kg levamisole HCl (Converge, MSD Animal Health).

6.2.7 Parasitological techniques

Deer samples were taken every two weeks for egg counts and larval counts. From cattle and sheep, faecal samples were taken every four weeks.

6.2.7.1 Faecal egg counts

Faecal egg counts (FEC) were undertaken at Massey University with the exception of InvAgR 2012 which were undertaken by AgResearch Grasslands. At Massey University the technique used was a modified McMaster technique where each egg counted represented 50 eggs per gram of faeces (eggs/g) (Stafford *et al.* 1994). At AgResearch the technique was similar but each egg counted represented 25 eggs/g.

6.2.7.2 Larval counts

Counts of lungworm larvae in faeces were undertaken at Massey University. These were estimated using a Baermann Technique where 4 g of faeces were suspended in water in a small measuring funnel for at least 12 hours (Henriksen 1965). The number of larvae recovered were counted and then converted to larvae/g faeces.

6.2.7.3 Worm counts

After slaughter of the tracer animals, the abomasa, small intestines, large intestines and lungs were removed and frozen at -20°C until processing for worm counts (Appendix 7 SOP 6 and 7). Individual organs were later thawed, and worm counts were undertaken on 5% aliquots of abomasal and small intestinal washings and 10% aliquots of large intestinal washings with the exception that only 2% aliquots were counted on five tracer deer with high burdens of *Ostertagia*-type nematodes in the abomasum at AgResearch Invermay in 2012. In addition, the abomasal tissue was subject to digestion in pepsin+HCL at 37°C until the mucosal tissue was disrupted, then 5% aliquots of these were also counted. All aliquots were sieved over a 37.5 µm mesh.

From the abomasum and small intestine, all available males for each genus up to a maximum of 50 per animal, were examined and identified to species. These proportions were used to calculate the worm burdens of each species within a genus for each animal. In the large intestine, for the identification of *Oesophagostomum* sp., all the females and males were collected and identified.

To count lungworms the perfusion method modified by Hoskins *et al* (2000b) was used and worm counts were performed on 100% of the recovered liquid. These were all assumed to be *Dictyocaulus eckerti*.

6.2.8 Statistical analysis

Statistical analyses were carried out using SAS (Statistical Analysis System, version 9.3; SAS Institute Inc., Cary, NC, USA).

6.2.8.1 Total number of anthelmintic treatments (AT)

This variable was analysed using the MIXED procedure with a linear mixed model that included the fixed effect of treatment and the random effects of year and location. Least squares means (LSM) and standard errors were obtained and used for multiple comparisons using the least significant difference test as implemented in SAS.

6.2.8.2 Time to receive first and second anthelmintic treatment

Statistical differences between experimental groups for cumulative numbers of animals receiving anthelmintic treatment within each location and year were evaluated with the Wilcoxon test of equality using the LIFETEST procedure. The inverse of survival curves for each experimental group were obtained using the Kaplan–Meier method (Kaplan and Meier 1958).

6.2.8.3 Tracer worm counts

Worm burdens were analysed after a logarithmic transformation using the MIXED procedure with a linear mixed model that included the fixed effect of treatment and the random effects of year and location. The LSM and standard errors were obtained and used for multiple comparisons using the least significant difference test as implemented in SAS. Results are presented as back-transformed LSM with 95% confidence intervals.

6.2.8.4 Live weight

Repeated measures of live weight on the same animals were analysed using the MIXED procedure with a linear mixed model that included the fixed effect of treatment and the random effects of year and location. Liveweight gain at the end of the period was analysed with a mixed linear model that included the fixed effect of treatment with year and location as random effects. Least squares means and standard errors were obtained for each treatment and used for multiple comparisons using the least significant difference test as implement in SAS.

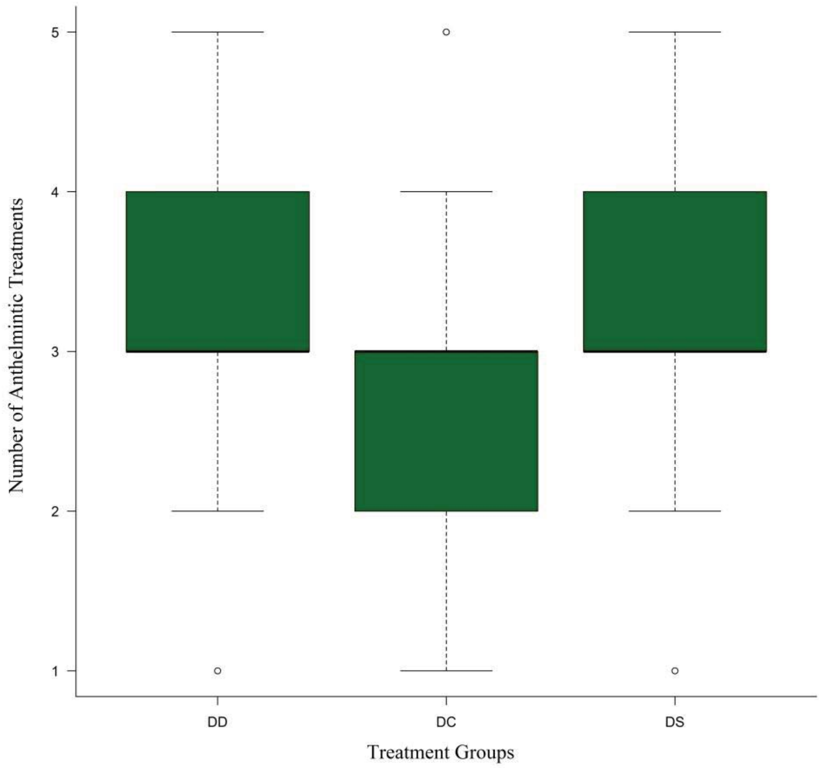
6.3 Results

6.3.1 Total Number of Anthelmintic Treatments (AT)

Deer were generally trigger-treated based on the weight criteria rather than egg or larval counts and none of the deer suffered from clinical parasitological disease.

A summary of the number of AT given is shown in Figure 0.2 and all the raw data is shown in Appendix 6, Section 1. The LSM of the number of AT per animal were DS=3.4, DD= 3.3, and DC=2.7. The numbers of AT received were significantly different between the treatment groups ($p<0.01$). A comparison of means between groups showed a significant difference in numbers between DD and DC ($p=0.0002$) and between DC and DS (p<0.0001) but there was no significant difference between DD and DS groups (p=0.51). The result excludes the first treatment that was given to all the treatments.

Figure 0.2 A box plot of the number of anthelmintic treatments given per animal by treatment group. DC = Deer with cattle; DD = Deer grazing alone; DS = Deer with sheep.



6.3.2 *Time to Receive First and Second Anthelmintic Treatment.*

Deer in DS and DD groups required treatment sooner than the DC group. A comparison of time to first anthelmintic treatment with Wilcoxon test of equality, between two groups, showed a significant difference between DD and DC (p<0.0001) and between DC and DS (p<0.0001) but there was no significant difference between DD and DS groups (p=0.83) (Figure 0.3). A comparison of time to second treatment showed a significant difference between DD and DC (p=0.0005) and between DC and DS (p=0.0004) but again there was no significant difference between DD and DS groups (p=0.68) (Figure 0.4).

Figure 0.3. Proportion of deer in each group to receive first anthelmintic treatment at each sampling period.

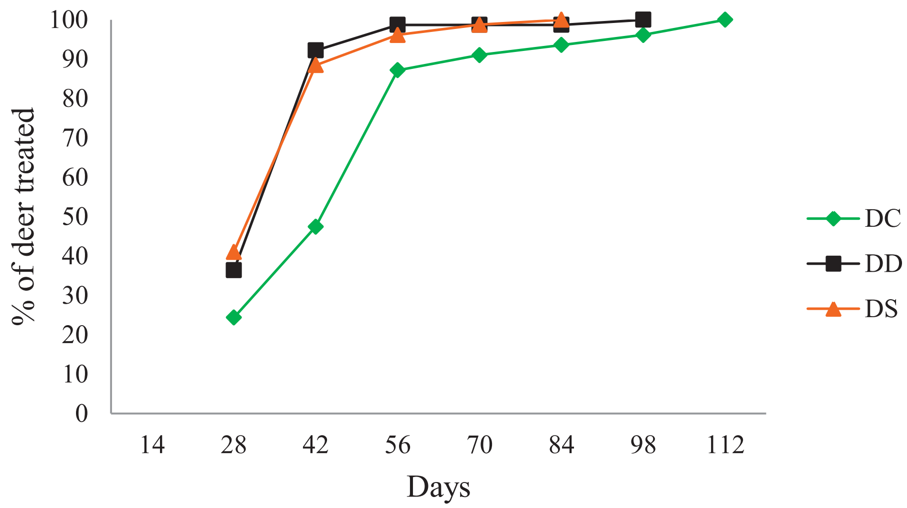
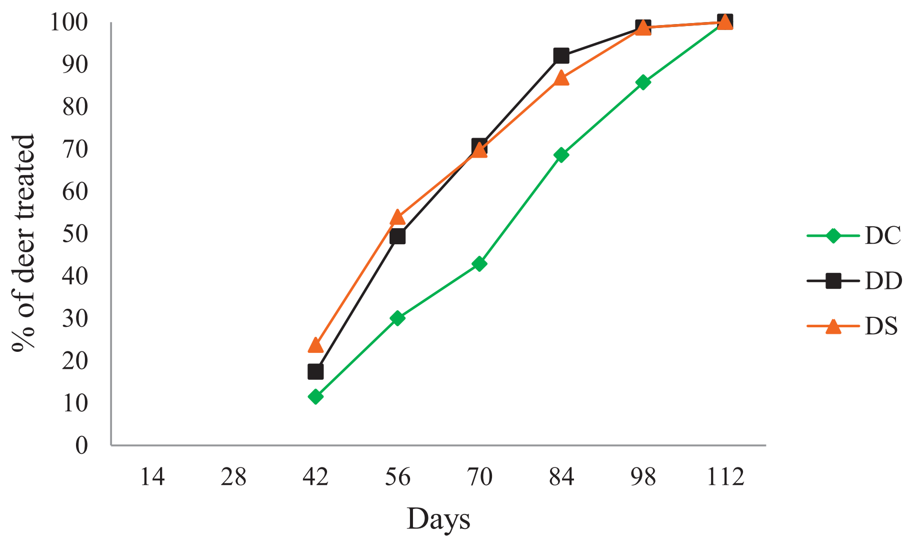


Figure 0.4. Proportion of deer in each group to receive second anthelmintic treatment at each sampling period.

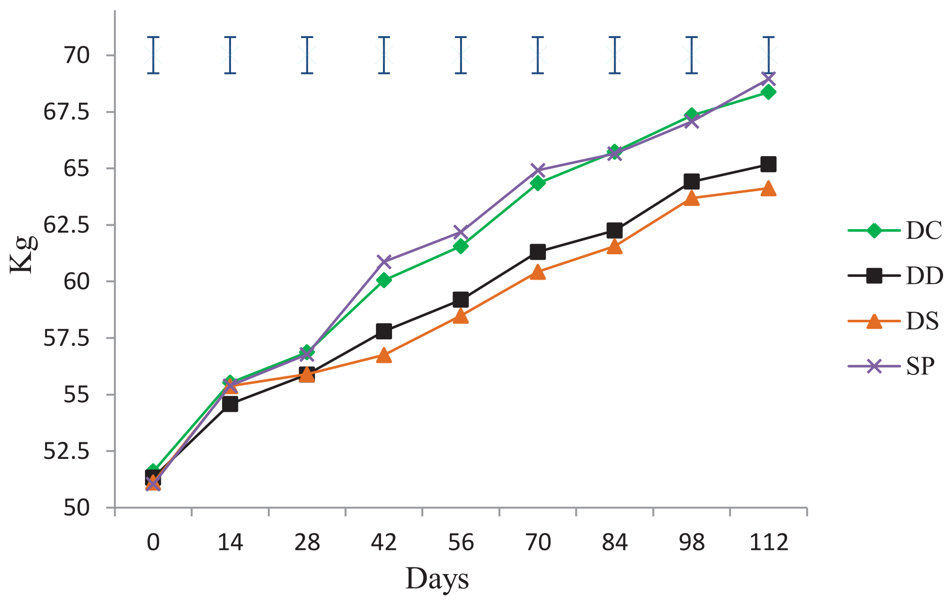


6.3.3 *Live weight and Liveweight gain:*

The LSM liveweight gains for the 16 week period, combined over both years and locations were SP= 17.9 Kg, DC= 16.6 Kg, DD=13.8 Kg and DS=12.8 Kg. The SP and DC groups had significantly higher liveweight gain ($p<0.0014$) than the other two groups. The live-weights followed the same trend (Figure 0.5). The LSM live weights at the end of 16 weeks (day 112), both locations and years combined, were SP= 61.4 Kg, DC= 61.3 Kg, DD=59.1 Kg and DS=58.6 Kg. The SP and DC groups had significantly higher live weights ($p<0.001$) than the other two groups.

The live weight and liveweight gain analysis will have been confounded by the number of treatments given to each of the groups.

Figure 0.5. Least square mean live weights for the different groups with SEM are shown.



6.3.4 *Faecal Egg and Larval Counts*

Raw data is found in Appendix 6 Section 2. Suppressive treatment of the SP group resulted in their egg counts consistently being 0 eggs/g.

6.3.5 *Tracer Deer Worm Counts*

6.3.5.1 Abomasum:

A summary of the overall abomasal *Ostertagia*-type worm burdens is shown in a boxplot in Figure 0.6 with the raw data in Appendix 6, Section.3. The LSM of the *Ostertagia*-type nematode values were DD=1950, DC=689, DS=370, SP=238. There were significantly more in DD than in DS (p=0.003) and SP (p=0.0002) but there was no difference in numbers between DC with DD (p=0.058) or with DS (p=0.056), nor between any other combination.

For *S. asymmetrica* LSM burdens were DD=117, DC=47, DS=9 and SP=26. Higher worm burdens were seen in the DD group than in the others but this was only significantly different when comparing DD with the DS group (p<0.01).

For *S. spiculoptera* LSM burdens were DD=148, DC=55, DS=13 and SP=32. Higher worm burdens were seen in the DD group than in the others but this was only significantly different when comparing DD with the DS group (p<0.01).

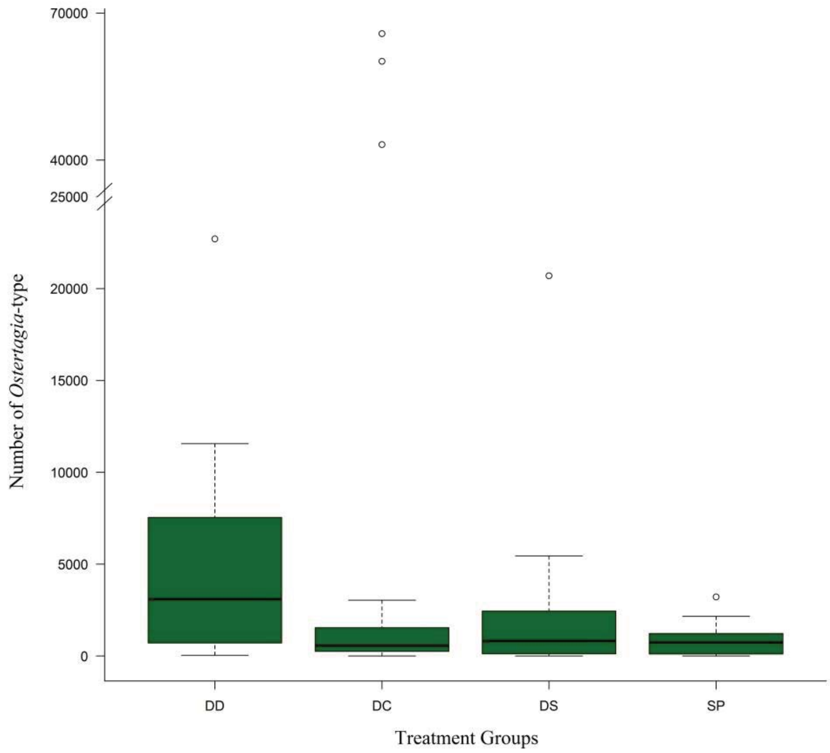
For *O. leptospicularis* LSM burdens were DD=886, DC=389, DS=137, and SP=264. Higher worm burdens were seen in the DD group than in the others but this was only significantly different when comparing the DD with the DS group (p<0.001). However, it approached significance for the difference between DD and the SP group (p=0.055).

For *T. circumcincta* LSM burdens were DD=2, DC=4, DS=35, and SP=2. Higher worm burdens were seen in the DS group and these were significantly higher than in the DD group (p<0.001), DC group (p<0.01) and the SP group (p<0.001). Nevertheless, the burdens were generally low in all groups.

For *O. ostertagi* very few nematodes were found in any animals. The LSM were DC=1, DD=0, DS=0 and SP=0. No statistical comparisons were made.

In 2012 in InvAgR, there was a notably high burden of *Ostertagia*-type nematodes in the second set of tracers in the DC group (LSM=56794) and they were dominated by *O. leptospicularis* (73%). This was higher than the burdens in all the other groups on this occasion.

Figure 0.6. Box plot of *Ostertagia*-type worm counts by group.

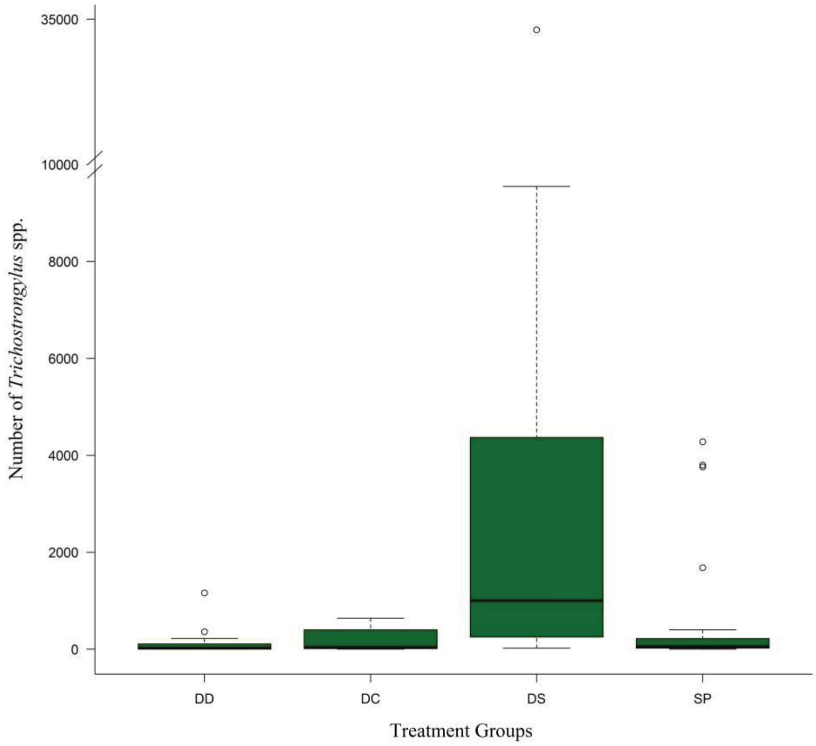


Trichostrongylus spp. abomasal worm burdens are shown in a boxplot in Figure 0.7. The LSM of the *Trichostrongylus* spp. nematodes values were DD=17, DC=37, DS=952, SP=54. There were significantly more *Trichostrongylus* spp. in DS than in the other groups (p<0.0001) with no difference between DD, DC and SP.

For *T. axei* LSM burdens were DD=54, DC=145, DS=1344, and SP=152. Higher worm burdens were seen in the DS group. Significant differences were found between the DS group and DC group (p<0.01), SP group (p<0.0001) and the DD group (p<0.01).

For *T. askivali* LSM burdens were low being DD=1, DC=1, DS=0, and SP=0 with positive worm burdens only being seen in the DD and DC groups. No statistical comparisons were made.

Figure 0.7. Box plot of abomasal *Trichostrongylus* spp. count by group.



Haemonchus contortus: This species was only found at PNmassey in low numbers and differences between groups were not significant p=0.087.

6.3.5.2 Small Intestine and Large Intestine.

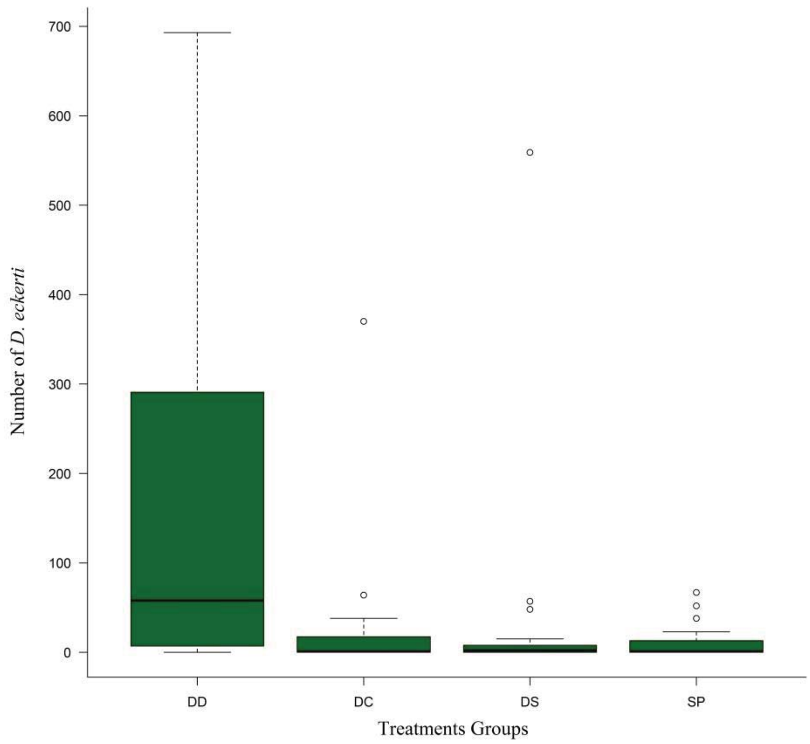
In the small intestine there were few nematodes in any group. There were negligible numbers of *Trichostrongylus (T. colubriformis* and *T. vitrinus)* and *Cooperia (C. punctata, C. pectinata* and *C. curticei)* in all the treatment groups. No significant differences were seen.

In the large intestine, *Oesophagostomum venulosum* was the only nematode found, the LSM values were DD=6, DC=6, DS=10 and SP=1 but there were no significant differences between groups (p= 0.1024).

6.3.5.3 Lungs

A summary of the lungworm burdens is shown in a boxplot in Figure 0.8. The LSM of the *Dictyocaulus eckerti* nematodes values were DD=40, DC=4, DS=3, SP=3 There were significantly fewer *Dictyocaulus eckerti* in DS, DC and SP ($p<0.0001$) than in DD treatment group.

Figure 0.8. Box plot of *Dictyocaulus eckerti* count by groups



6.4 Discussion

The results of this study have demonstrated that for young deer in the 16 weeks after weaning, cross-grazing with an alternative ruminant species offers some advantages over mono-grazing. However, the advantages varied between the use of sheep or cattle and in the ability to control different species of parasites. The DC group received fewer treatments than other groups and grew as well as the SP group, indicating that, within the constraints of this grazing study, cross-grazing with cattle was the most effective way to graze deer to obtain additional parasite control. Consistent with the fewer treatments received was the observation that the time for DC deer to receive their first and second treatments was also slower than for the other groups. Nevertheless, all deer did receive at least one treatment, and most a second treatment, indicating that cross-grazing alone was not sufficient to fully control nematodes in young deer in the conditions existing in this study. By comparison the DS group required the same number of treatments as the DD group and still grew less well than the SP group. There were advantages for the DS group in that they had fewer *Ostertagia*-type nematodes than DC and DD.

The lungworm, *Dictyocaulus eckerti*, has historically been considered to be the most important internal parasite of farmed deer in New Zealand, especially early in autumn (Charleston 1980; Mason 1985a) because it can cause death in young animals. In this study, the highest burdens were found in deer in the DD group. Another important clinical result was that cross-grazing with either sheep or cattle was effective in achieving a high level of control of this parasite. This is consistent with earlier reports of low cross-infection of *D. viviparus* from cattle to deer and no cross-infection of *D. eckerti* from deer to cattle (Johnson *et al.* 2003b). In addition, it was observed that some tracer deer in the SP group had small burdens of lungworm, reflecting survival of larvae on pasture prior to the commencement of the study. Since it is known that *D. viviparus* is capable of overwinter survival (Gupta and Gibbs 1970; Strube *et al.* 2007; Laabs *et al.* 2012), it is likely that *D. eckerti* may do so as well.

Of the gastrointestinal nematodes, the *Ostertagia*-type nematodes of deer are considered to be the most important because of their high prevalence. In this grazing experiment the highest burdens were found in the DD group which was significantly higher than for the DS and SP groups, indicating a reduction of the burdens of *Ostertagia*-type nematodes resulting from grazing with sheep. Even though the difference between the DD and DC groups was not significant, results showed a tendency toward lower counts in the DC groups compared to DD groups (p=0.058). Thus cross-grazing with sheep appears to be more effective in reducing the challenge of *Ostertagia*-type nematodes for deer than cross-grazing with cattle.

Of the *Ostertagia*-type nematodes, *O. leptospicularis* was the most prevalent species in all the treatments groups with the highest numbers recovered from tracer deer in the DD group but this was only significantly higher than the DS and SP groups, not the DC group. This particular species is of interest as it is known to be able to readily parasitise cattle (Bisset 1980; Borgsteede 1981; Buel *et al.* 1984; McKenna 2009a) and also sheep (Borgsteede 1981; McKenna 2009a). Whilst the DD group had the highest counts it was notable that the second set of tracers at InvAgR had high burdens in the DC group in 2012 which were higher than the DD group at that time. The reason for this high number is uncertain but may involve anthelmintic resistance to the double dose of oxfendazole used to treat the tracer deer at this location at that time although it was not observed for the other tracer sets.

As expected, tracer deer in the SP group had the lowest burdens of *Ostertagia*-type nematodes, but these were not significantly lower than in the DS group further demonstrating the value in cross-grazing with sheep. Since the faecal egg counts in the SP group were consistently zero throughout the study period the presence of small burdens in the tracers in the SP group would indicate that some larvae had survived on pasture from before the study began. The absence or very low burdens of *T. circumcincta* and *O. ostertagi* from the tracer deer in any group is consistent with the results from the parallel challenge studies conducted at Massey University, which showed that the establishment rate of these in deer was low (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a; Tapia-Escárate *et al.* 2015b;Chapter 5).

Abomasal *Trichostrongylus* spp. counts were significantly higher in the DS groups, compared to all the other groups implying that *T. axei* established more successfully in sheep than the other host species. Parallel challenge studies of the infectivity of cattle and sheep nematodes for deer indicate that the establishment rate of *T. axei* in sheep was 74% compared to 12% in deer when sheep origin larvae were used (Tapia-Escárate *et al.* 2015b; Chapter 5) and when cattle origin larvae were used there were no significant differences in the establishment rate of *T. axei* between cattle (19%) and deer (25%) when using cattle-origin infective larvae (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a). Thus the results from the present study are consistent with those cross-infection studies. In two field studies in Australia contrasting results were seen with *T. axei* burdens in sheep where one found lower counts in sheep as a result of cross grazing with cattle (Barger and Southcott 1978) whilst the second found higher counts when co-grazing with cattle (Arundel and Hamilton 1975). According to Ross and Purcell (1969) different strains of *T. axei* have a predilection for different host species. In the DS in this study group, *T. axei* was cycling relatively easily, suggesting that in these paddocks sheep and deer were susceptible to this *T. axei* strain and this may be at least part of the explanation for the higher *T. axei* counts in deer in the DS group.

Abomasal *Haemonchus contortus* were only seen at PNmassey. Numbers were generally small and the differences between groups were not significant. This species has been observed to establish in deer at a lower rate than in sheep but at a similar rate to cattle (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a; Tapia-Escárate *et al.* 2015b; Chapter 5). As there were only small numbers it is unlikely they would have been having a significant clinical impact on deer.

The choice to use a growth rate of only 80% of the SP group as a trigger to treat animals in the other groups was determined by analysing results from an earlier study with young deer at Massey University during the same months of the year, where a group of animals were trigger treated based on faecal egg and larval count criteria. The aim in the present study was to set a level that would be sensitive to lower growth rates. This trigger criterion was successful because for the majority of cases the weight gain criterion was the reason that animals were treated. Growth rate is a very important measure because it has economic implications. In comparison the trigger based on faecal egg and larval counts was only used on a few occasions and none of the deer suffered from parasitological clinical disease. This is consistent with other work in deer that shows that weight is a more sensitive marker of parasites than FEC or FLC (Hoskin *et al.* 2000b; Hoskin *et al.* 2007).

It needs to be acknowledged that live-weight data analysed here are potentially confounded by the differences in numbers of anthelmintic treatments given. It may also be affected by differences in pasture residual dry matter values between sheep, cattle and deer which can influence the available pasture for deer the next time they grazed paddocks, despite that attempts were made to minimise this effect by measuring pasture dry matter, and adjusting stocking rates accordingly. Nevertheless, the DC group grew as well as the suppressively treated SP group suggesting advantages in terms of growth rate for this particular model of cross-grazing. The deer in the DS group received more anthelmintic treatments but still grew less well than either the DC or SP groups. Together with the parasitological effect, the growth effect may also be caused by the feeding habits of deer that can be complemented by cattle whilst being competitive with sheep (Hoskin 2007).

At PNmassey the liveweight gains achieved in the DC and SP groups (19 g/d) were similar to the industry maximum performance targets for autumn for New Zealand red deer (18.35 g/d) (Stevens 2014). This would indicate that together with the grazing management the use of supplementary feed on top of the available pasture was more than adequate to allow all the treatment groups animals to grow at a rate consistent with accepted industry standards.

This study did not consider the effects of cross-grazing on the young sheep or cattle. Both groups were regularly treated at four weekly intervals and because of drought conditions in both years, particularly at Massey University, they were given supplementary feed which would have distorted any effect of deer creating unsuitable pasture for cattle in particular. Future studies should also consider the effects of cross-grazing on these animals and also any benefits from cross-grazing with both cattle and sheep simultaneously.

In summary, this study demonstrated that cross-grazing with cattle, in particular, was reasonably effective in reducing the size and the effects of gastrointestinal nematode burdens in the young deer and that cross-grazing with cattle or sheep in the autumn was effective in reducing the lungworm numbers in weaner deer. Based on the criteria applied, cross-grazing did not provide sufficient control to completely avoid use of anthelmintic treatments in either the DC or DS groups, but deer in the group cross-grazing with cattle required fewer treatments than the DD group. In principle, fewer treatments should reduce the rate of development of anthelmintic resistance.

Chapter 7 General Discussion

3.3 Introduction

The aim of this thesis was to better understand different aspects of gastrointestinal nematode (GIN) infection in red deer, including pathogenicity, diagnosis and control. In New Zealand, parasites are an important economic and clinical problem (Audigé *et al.* 1998) and they have been acknowledged as a problem since the commencement of deer farming (Mason 1977; Watson and Charleston 1985b; Audigé *et al.* 1998). The most important parasites in farmed deer are the lungworm *Dictyocaulus* spp. that can cause severe disease in naïve animals (Charleston 1980; Mason 1985a) and GIN. Due to the initial view that GINs were of limited importance, few studies were undertaken with these parasites alone. However, the impact of GIN has likely been underestimated and recent evidence of widespread resistance in the *Ostertagia*-type nematodes to ML anthelmintics (Lawrence 2011; Lawrence *et al.* 2012; Hodgson 2013; Lawrence *et al.* 2013; Mackintosh *et al.* 2013) and evidence of resistance to oxfendazole in these same nematodes (Lawrence *et al.* 2013) has increased the likely importance of this group of nematodes.

The first studies (Chapter 2 and 3) in this thesis investigated the production impact of GIN in deer with a focus on the *Ostertagia*-type nematodes because of their importance in deer production. The second studies (Chapter 5 and 6) were undertaken because while it is known that red deer can be infected with some GIN of cattle and sheep, it was important to understand the cross-infection risks associated with mixed species grazing systems and also to explore the effectiveness of an organised cross-grazing system with sheep and/or cattle for controlling deer nematode parasitism. Finally, as there was little specific information on the prevalence of different species of nematodes in deer in New Zealand, including species for which the preferred hosts are sheep or cattle, one of the milestones of this thesis was to develop and consolidate PCR methods to correctly identify parasites to species level in live animals and to gather information about the prevalence of deer nematodes in New Zealand.

3.4 GIN in deer in New Zealand.

3.4.4 *Ostertagia-type nematodes*

This thesis supports the evidence that deer-specific nematodes in the sub-family Ostertaginae (=*Ostertagia*-type; *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera* and *Ostertagia leptospicularis*) are arguably the most important GIN in deer. Considering this collective group of species together as one there are a number of general observations

In the cross-grazing study (Chapter 6) the tracer animals were exposed to a natural challenge of nematodes for five weeks and the highest burdens of deer-specific *Ostertagia*-type nematodes were observed in the deer which were mono-grazing ("DD" group). Similarly, in the survey on identification and distribution of GIN in New Zealand study (Chapter 4), the highest prevalence of deer *Ostertagia*-type nematodes was found on farms that grazed only deer (Deer only). Therefore, it can be inferred that the introduction of cattle or sheep in the system can assist in the reduction of the number of *Ostertagia*-type nematodes in deer. However, the advantages varied between the use of sheep or cattle with regards to the level of control of different species of parasites.

For control of *Ostertagia*-type nematodes the inclusion of sheep appears to have some advantages. As shown in the cross-grazing study (Chapter 6) the DD group was most exposed to *Ostertagia*-type nematodes whilst deer cross-grazing with lambs (DS) had significantly fewer *Ostertagia*-type nematodes. Similarly, in the survey on nematodes in deer (Chapter 4), farms that included deer and sheep (Deer/Sheep) had significantly fewer *Ostertagia*-type nematodes than the farms with only deer. In comparison, although there was a tendency toward lower counts of *Ostertagia*-type species in the deer grazing with cattle group (DC) compared to the DD group, this difference was not significant, likely because three tracer deer at AgResearch Invermay in 2012 in the DC group, had very high burdens of *Ostertagia*-type nematodes (n= > 40,000), affecting the total results. This result may reflect the ability of *O. leptospicularis,* one of the *Ostertagia*-type species, to also readily parasitise cattle but it was not possible to decisively conclude the actual reason in that study – see below. However, in the survey study (Chapter 4), farms that included deer and cattle (Deer/Cattle) also had significantly fewer *Ostertagia*-type nematodes than the farms with only deer which is a slightly different trend to that seen in the cross-grazing study (Chapter 6). Clearly further study is required to assess the risk of cross infection between cattle and deer for *Ostertagia*-type parasites.

Considering the members of this group of nematodes species by species some different trends are apparent. In tracer deer in the cross-grazing study, *O. leptospicularis* was the most prevalent species in all the treatments groups. To the contrary in the survey study (Chapter 4), this nematode was the least common of the three deer *Ostertagia*-type species although only significantly different from *S. asymmetrica* which was the most prevalent. In the initial pathogenicity study (Chapter 2) which was also undertaken using a mixed culture of larvae from young weaner deer from the Massey University Deer Unit, *O. leptospicularis* was also the least frequent. The reason for these different observations isn't clear but may have an element of seasonality affecting which species dominates at times. As indicated above, *O. leptospicularis* is of interest as it is known to be able to readily parasitize cattle (Bisset 1980; Borgsteede 1981; Buel *et al.* 1984; McKenna 2009a). However, it is also described that it can infect sheep (Borgsteede 1981; McKenna 2009a) although it appears to be rarely reported from sheep. In the survey study (Chapter 4) a higher prevalence of this species was seen in the Deer-only farms, but this prevalence was only significantly higher than Deer/Sheep farms, and not significantly different than Deer/Cattle farms and those with all three host species "DSC" (Deer/Sheep/Cattle). Similarly, in the cross-grazing study, the only significant differences were seen between the DD and DS group. These results suggest that, even if sheep can be infected with *O. leptospicularis*, cattle are more likely to preserve the cycle of this species on the farm.

In the cross-grazing and in the survey study higher prevalences of *S. asymmetrica* and *S. spiculoptera* were observed in the Deer-only farms compared with those with deer grazing with an alternative host, but significant differences were not always found. These results suggest that grazing with an alternative host can aid in the control of these two nematode species.

Ostertagia-type nematodes which have cattle or sheep as their normal hosts, may also be found in deer but usually in very low numbers. *Ostertagia ostertagi,* which has cattle as its preferred host, was found in low numbers in deer in the cross-grazing study

(Chapter 6). This is supported by an indoor infectivity trial where deer infected with GIN from cattle and where the establishment rate of *O. ostertagi* was only 0.7% in deer compared to 31.0% in cattle (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a). Similarly, for *T. circumcincta* for which the normal host is sheep, a low prevalence, small burdens and low establishment rate were observed in the survey, cross-grazing and indoor infectivity studies respectively. Although no studies were conducted in sheep and cattle to verify that GIN were reduced when cross-grazing with deer, the low infectivity of *O. ostertagi* and *T. circumcincta* in deer suggest that cross-grazing sheep and cattle with deer may also be a good tactic to controlling these nematodes in these other domestic species.

Because of the known importance of the deer *Ostertagia*-type nematodes, one of the aims of this thesis was to undertake a pathogenicity study (Chapter 2) in which the intention was to infect the deer with three doses of *Ostertagia*-type nematodes to cause an effect on growth rate but without overt clinical signs. However, while the dose of deer-origin infective larvae comprised 40% *Ostertagia*-type, a high proportion of *Oesophagostomum* spp. larvae (53%) were also present. As a result, the animals were clinically affected by the large intestinal lesions associated with *Oesophagostomum* spp. and this study could not effectively investigate the effect of different dose rates of *Ostertagia*-type parasites *per se*.

3.4.5 *Oesophagostomum spp.*

Oesophagostomum venulosum is commonly found in the large intestine of red deer in New Zealand, even though its preferred hosts are sheep and goats (Hoskin *et al.* 2000b; McKenna 2009a). *Oesophagostomum sikae* which is morphologically similar to *O. radiatum* has been found in sika deer (Cameron and Parnell 1933; in Popova 1965) and in red deer in New Zealand (Chapter 2).

In sheep, *O. venulosum* is not considered pathogenic, principally because it does not induce nodule formation around the larvae within the mucosa. However, nodule formation has been reported in at least two studies with experimentally infected sheep (Goldberg 1952; Clark *et al.* 1978). In comparison, cattle do develop a pronounced inflammatory nodule around *O. radiatum* larvae within the mucosa and a large number of nodules can cause considerable damage, resulting in clinical disease (Taylor *et al.* 2007). To date there are no reports on the pathogenicity of *O. sikae* in any deer species.

As explained previously, the pathogenicity initial study was terminated earlier than planned for the onset of clinical signs, due to the occurrence of numerous inflammatory nodules in the mucosa of the terminal small intestine and large intestine together with substantial oedema and a general inflammatory response around the large intestine, likely due to the present of a large quantity of *Oesophagostomum* spp. nematodes. In that study, *O. venulosum* was the predominant species but a small proportion of the newly recorded *Oesophagostomum sikae* were also identified. Because the overall establishment for *Oesophagostomum* spp., based on counts of adult nematodes was low (9-13%) the degree of pathological damage seen in this study was not expected. It remains unclear if this was caused by *O. venulosum* larvae trapped within the mucosa subsequently forming inflammatory nodules or is a normal consequence of *O. sikae*. It was intended that the larvae would be recovered from the mucosa of the large intestines with pepsin digestion, but this procedure was not effective at releasing the larvae and the identification of the larvae to species level was not possible. Because there are no reported cases that described nodules in the large intestine of deer by either *O. venulosum* or *O. sikae* only assumptions can be described. If it is assumed that *O. sikae* is more likely to induce mucosal nodules as for most other *Oesophagostomum* species such as *O. radiatum,* then many of the larvae given to these deer may have remained within the mucosa. However, in the pathogenicity follow-up study (Chapter 3) *O. sikae* was also present but no nodules were found. In addition, in the survey study (Chapter 4), the prevalence of this nematode was low although it was still identified on 17% of the farms and there have been no reports of nodules associated with these parasites described in New Zealand. In the cross-grazing study (Chapter 6), somewhat surprisingly. *O. sikae* was not identified in any deer and the burdens of *O. venulosum* were low and no nodules were observed. Therefore, it is concluded that high infections with *Oesophagostomum* spp. were clearly the cause of the occurrence of numerous inflammatory nodules in the mucosa of the terminal small intestine and large intestine but it remains unclear as to which species was involved. It was also very obvious from this study that this challenge caused considerable pathological damage with a resultant rapid reduction in weight gain and voluntary feed intake.

In the survey study (Chapter 4) larvae from the *O. venulosum* species was the most prevalent at farm level and in overall frequency. The overall prevalence was 47%. Similarly, a high percentage of larvae of this nematode (53%) was recovered from six Massey University-born weaner red deer which were the source of the larvae given to the deer in the initial and follow-up pathogenicity studies (Chapter 2 and 3). Even though its normal host is considered to be sheep, this species is consistently present in studies in red deer in New Zealand although with different frequencies (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b; Hoskin *et al.* 2005). The frequency in the survey study (Chapter 4) was even higher on farms that also grazed sheep compared to deer-only farms. No significant differences between groups (p= 0.1024) in the burdens of *O. venulosum* were observed in the cross-grazing study (Chapter 6) but relatively greater burdens of this nematode were recovered in deer that were cross-grazing with sheep. Such results suggest this species is well adapted for deer. However, in the related cross-infection study (Tapia-Escárate *et al.* 2015b; Chapter 5) which utilised sheep-origin infective larvae, the establishment rate of *O. venulosum* was significantly lower in red deer than in sheep but 5.8 % of the infective dose still established compared to 21.6 % in sheep. This suggests that sufficient larvae must still establish in deer to allow the infection to carry forward on deer pastures even though it is less well adapted to deer than for sheep, its natural host. The results can be somewhat distorted when it is considered that *O. venulosum* is highly fecund (Koprivnikar and Randhawa 2013) and the frequency of this nematode in Chapters 2, 3 and 4 are likely to overestimate the actual worm burdens. In a second study, not part of this thesis, when using cattle-origin infective larvae to infect cattle and deer (Tendoesschate *et al.* unpublished;Tapia-Escárate *et al.* 2015a), low burdens of *O. venulosum* were present in the infective dose, but this nematode established better in deer than in cattle (p = 0.016). *O. venulosum* is not considered a normal parasite of cattle (Borgsteede 1981) which is consistent with this finding. Even if cross-infection of *O. venulosum* between sheep and deer is successful, this nematode is considered of limited pathogenic significance under natural conditions.

The distribution of *O. venulosum* in Australia has been described as more common in winter-rainfall areas (Cole *et al.* 1986) but results from this study under New Zealand conditions indicate it would be considered a "normal" deer parasite observed equally commonly in both islands. In lactating ewes in New Zealand, it was observed to be similarly common across a range of farm types and locations (Hervé *et al.* 2003). Whilst other species from this genus, including *O. radiatum*, *O. venulosum* and *O. columbianum* struggle in areas with cold winters (Sutherland and Scott 2009), the free-living stages of *O. venulosum* are able to survive on pasture over winter in temperate areas (Taylor *et al.* 2007) so it is not unexpected that it is reasonably common in New Zealand. A somewhat surprising result in the survey study (Chapter 4) was the higher prevalence of *O. venulosum* in winter. However, as most of the samples from DS, where the highest prevalence of this nematode was found, were taken in winter (144 of 168 larvae) the results may have a bias due to the time of collection of samples.

3.4.6 *Abomasal Trichostrongylus spp.*

Trichostrongylus axei and *Trichostrongylus askivali* are the two abomasal *Trichostrongylus* species commonly found in deer in New Zealand.

Trichostrongylus axei has been recorded from a range of grazing species and it was considered as the only member of this genus in the abomasum of red deer until 2009 when *T. askivali* was identified in deer from Massey University in an unpublished study (McKenna 2009c). This recent discovery of *T. askivali* in New Zealand means we know very little about it in terms of anthelmintic efficacy and pathogenicity. This species was originally described from red deer in Scotland (Dunn 1965) so its presence in New Zealand is not surprising. The total overall prevalence in New Zealand (Chapter 4) was low (3%), but the farm level prevalence throughout in New Zealand was 32% (Chapter 4) indicating how common it is in New Zealand and that most likely it was misidentified before 2009 because of the morphological similarities between these two abomasal *Trichostrongylus* species. This nematode was also found in the cross-grazing study (Chapter 6) and in the first stage of the pathogenicity initial study (Chapter 2) but in low numbers in both studies.

T. axei is a species which is capable of infecting a variety of hosts with little host preference (Borgsteede 1981) including deer. The pathogenicity of *T. axei,* as for other trichostrongyloids, is influenced by the number of nematodes present and by the development of the host immune response. The clinical signs reported in lambs include diarrhoea, depression in food intake and weight loss. In severe cases, it can even cause death (Gibson 1954b, 1954a, 1955). In the cross-grazing study (Chapter 6) high burdens of *T. axei* were found, particularly in the tracer deer in the DS groups. This coincides with the lower liveweight gains and frequently used anthelmintic treatment for deer in this group implying that higher burdens can generate production losses in farmed deer too. However, although there is this relationship it is not necessarily causal as it is confounded by the presence of other GIN species.

Parallel studies on the infectivity of cattle and sheep nematodes in deer (Tapia-Escárate *et al.* 2015a; Tapia-Escárate *et al.* 2015b; Chapter 5) indicate that the establishment rate of *T. axei* was higher in sheep (74%) than in deer (12%) when sheep-origin larvae were used (Tapia-Escárate *et al.* 2015b; Chapter 5) again suggesting that at least this provenance of *T. axei* was better adapted for sheep. Interestingly, in the second study investigating the establishment of cattle origin nematodes in deer (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a), the *T. axei* establishment rates observed in both the deer and cattle were lower than those for sheep observed in the first study and interestingly there was no significant difference in the establishment rate of *T. axei* between cattle (19%) and deer (25%). Overall, these cross-infection results suggest that *T. axei* establishes better in sheep than in any other species but this doesn't take other factors into account.

Although the results indicate that *T. axei* of cattle origin appeared to establish more successfully in deer the two studies were undertaken at different times and many factors including the mix of species given as well as the host immune response may have varied and influenced the results. In contrast, in the survey study (Chapter 4), the prevalence of *T. axei* on Deer/Cattle farms (8%) was significantly higher than for any other combination implying that the presence of cattle in the contamination of pasture was increasing the prevalence of *T. axei* in deer. Results from published studies give similar contradictory results for infections in sheep in two field studies in Australia. In one, lower *T. axei* burdens were found in sheep as a result of cross-grazing with cattle (Barger and Southcott 1978) whilst a second found higher numbers when co-grazing with cattle (Arundel and Hamilton 1975). According to Ross and Purcell (1969), different strains of *T. axei* have a predilection for different host species, and this may be the reason for the variations between different studies.

3.4.7 *Haemonchus contortus*

Haemonchus contortus have been found in a range of ruminants including deer although their preferred hosts are considered to be sheep and goats. Their pathogenicity is mostly due to their hematophagous feeding behaviour (Holmes 1987) and the consequences are animals in poor condition with anaemia. In the North Island it is well known to be capable of killing sheep and goats but there are only anecdotal comments from clinicians reporting haemonchosis in red deer (Swanson *et al.* 2007).

The establishment rate for *Haemonchus* larvae observed in the initial pathogenicity study was 31-51% (Chapter 2) which was higher than that observed in the two cross-infection studies in sheep (Tapia-Escárate *et al.* 2015b; Chapter 5) or cattle parasites (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a) into red deer, where the establishment rates of *H. contortus* was in red deer were 11%, and 19% respectively. An explanation for this difference could be that *H. contortus* of deer origin are more adapted for deer and hence establish in them more successfully than those of sheep or cattle origin. Alternatively. it could reflect the deer in the two latter studies were pasture reared and had already had some exposure stimulating some measure of immunity even though they were of similar ages in each study.

In deer the low prevalence of *Haemonchus* in the survey study (Chapter 4) coincides with other studies in New Zealand where this nematode has been reported with low burdens in deer (Hoskin *et al.* 2000a; Hoskin *et al.* 2000b) including the cross-grazing study (Chapter 6). In part, this will reflect the time of sample collection for the survey as *Haemonchus* is more likely to be common during the warmer summer and early autumn months. Overall it is known that this nematode prefers warm tropical and subtropical areas because larval development requires relatively high temperatures and humidity. However, given the observed establishment rates in the cross-infection studies (Tapia-Escárate *et al.* 2015a; Tapia-Escárate *et al.* 2015b; Chapter 5) and the initial pathogenicity study (Chapter 2), the results suggest that under field conditions sufficient burdens of *H. contortus* could build up in deer to be clinically significant if the environmental conditions are favourable.

3.4.8 *Small intestinal GIN.*

Very few nematodes were found in the small intestine of deer in all studies undertaken. In the initial pathogenicity study (Chapter 2) there were negligible numbers of *Trichostrongylus (T. colubriformis* and *T. vitrinus)* and *Cooperia (C. punctata, C. pectinata)* in all the treatment groups. In the cross-infection study (Tapia-Escárate *et al.* 2015b; Chapter 5), no *Trichostrongylus colubriformis* or *Trichostrongylus vitrinus* were seen in any deer but were present in all sheep. In the cross-grazing study (Chapter 6) insignificant numbers of any small intestinal GIN species were found with only a few *Trichostrongylus* spp. (*T. colubriformis* and *T. vitrinus)* and *Cooperia* spp. (*C. punctata, C. pectinata* and *C. curticei)* being found. In the survey study only *T. vitrinus, C. curticei* and *C. oncophora* were identified (Chapter 4). Of these the most common was *C. oncophora* with a 4% prevalence overall and was found on 17/59 properties indicating it had a very low prevalence even though it was found on many properties. The nematode *T. vitrinus* was found with a 3% of prevalence overall and was found on 17/59 properties with the prevalence of *C. curticei* being < 1%. The prevalence found for *C. oncophora* (4%) in the survey study (Chapter 4) is in contrast to the results of the cross-grazing study (Chapter 6) and pathogenicity initial study (Chapter 2) where this species wasn't found at all. In a related cross-infection study utilizing cattle-origin infective larvae (Tendoesschate et al. unpublished;Tapia-Escárate *et al.* 2015a) the establishment rate of *Cooperia* spp. in cattle was 72.0% compared to only 2.3% in deer but when considered at the species level there was an even lower establishment rate of *C. oncophora* (<1%) in deer. Overall, these results imply that *C. oncophora* is an insignificant parasite for deer. A similar poor ability of both *T. vitrinus* and *C. curticei* to establish in deer indicates that they are not important parasites for deer.

3.4.9 *Dictyocaulus spp.*

The lungworm, *Dictyocaulus* spp., is generally considered to be the most important internal parasite of farmed deer in New Zealand, especially early in autumn (Charleston 1980; Mason 1985a). In the various studies for this thesis, *Dictyocaulus eckerti* was only seen in the cross-grazing study since these animals were naturally infected. Interestingly, cross-grazing with either sheep or cattle was effective in achieving a high level of control of this parasite. This is consistent with earlier reports of low cross-infection of *D. viviparus* from cattle to deer and no cross-infection of *D. eckerti* from deer to cattle (Johnson *et al.* 2003b). To date, there are no reports of cross-infection between *D. filaria* from sheep to red deer and it is considered unlikely to occur.

D. eckerti is generally considered to be less robust on pasture than many other strongylid nematodes but under certain conditions of low temperatures some L3 of *D. viviparus* are capable of overwinter survival (Gupta and Gibbs 1970; Strube *et al.* 2007; Laabs *et al.* 2012). It would appear from results of the cross-grazing study (Chapter 6) this likely applies to *D. eckerti* as well, since some tracer deer in the suppressively treated group still developed small burdens of lungworm even though the pastures they were grazing on had been grazed by sheep and cattle for the period immediately prior to the study commencing. It is presumed this reflects the survival of larvae on pasture prior to the commencement of the study.

3.5 Diagnostic Techniques for GIN

A good diagnostic technique is essential for good control of parasites, as clinical signs of helminth disease are not very specific. It is also important for detection of subclinical infections and resistance to anthelmintics.

3.5.4 *Nematode Burdens*

The morphological methods for the identification of adult stages to species level can only be used at post mortem. Because of its reliability this method was used as the preferred method in the pathogenicity studies (Chapter 2 and 3), cross-infection study (Tapia-Escárate *et al.* 2015b; Chapter 5) and cross-grazing study (Chapter 6).

In live animals in the pathogenicity studies (Chapter 2 and 3), cross-infection study (Tapia-Escárate *et al.* 2015b; Chapter 5) and the cross-grazing study (Chapter 6) quantifying eggs in faeces was the main indirect method for estimation of worm burden. The deer in these studies were young animals after weaning. The relationship between egg counts and worm burdens in deer is considered to be poor in one-year-old deer (Mackintosh and Tolentino 2009), but in a study on anthelmintic efficacy by Mackintosh *et al.* (2014b) it was found that there was a high correlation between *Ostertagia*-type egg output and worm burden in younger deer. A similar change in the relationship with a developing immune response in the young deer was reported when this correlation was shown to be moderate in young animals in their first autumn, but low in following spring and summer when the animals were older (Mackintosh *et al.* 2014a). All the studies for this thesis were with young animals during the period when it could be expected the correlation between egg counts and worm burdens was reasonable except for the survey study (Chapter 4) where samples from older animals were used. In this latter study, faecal samples were cultured and the larvae then identified. The change in the relationship between egg counts and worm burdens may have influenced these results and probably resulted in the *Ostertagia*-type nematodes being underestimated for their true prevalence.

3.5.5 *Blood Parameters*

In the pathogenicity studies (Chapter 2 and 3) the aim was to find markers, including blood, serum biochemical and haematological parameters to estimate abomasal worm burdens in deer with subclinical parasitism. Though in the initial pathogenicity study (Chapter 2) the deer were clinically affected by the large intestinal lesions associated with *Oesophagostomum* spp., the study, while providing useful observations about *Oesophagostomum,* could not achieve the aim of investigating subclinical effect of *Ostertagia*-type nematodes. The blood test results in this study (Chapter 2) reflect a classic parasitology response in the infected deer related to damage to the gut with a rapid turnover rate of cells, a marked serum albumin decrease, globulin increase and a decrease in the albumin to globulin ratio. Similar trends have been associated with *Ostertagia ostertagi* in cattle (Murray *et al.* 1970) and with *Teladorsagia circumcincta* in sheep (Coop *et al.* 1982), but also with heavy infections with *Oesophagostomum venulosum* in sheep (Badrie and Kamenov 1982). In addition, there was a significant increase related to infective dose rate in the eosinophil and basophil counts. It is well known that eosinophils and basophils are associated with an immune response against helminth infections mostly linked with Type 2 immune responses. In the follow-up pathogenicity study (Chapter 3) with lower infective doses of infective larvae there were no significant differences in voluntary feed intake or growth rates, and also no significant difference in the levels of albumin, but there was a significant increase in the levels of globulin and a decrease in the albumin to globulin ratio in the infected deer. In addition, there was a significant increase in the eosinophil and basophil counts in the infected animals. These latest results suggest that there was less damage and lower albumin losses through the mucosa, but still an immune response against the nematodes. In general, as for sheep and cattle, these changes are very non-specific and not likely to be helpful in diagnosing subclinical infections in particular.

No significant differences were seen in the pepsinogen levels between the different treatments in both the initial pathogenicity study (Chapter 2) and the follow-up pathogenicity study (Chapter 3). There have only been a few studies that have measured pepsinogen levels in deer and they have found conflicting results. Audigé *et al.* (1998) found that pepsinogen had an inverse relationship at herd level related to summer growth of weaners, but this relation was not proven at the individual animal level. In addition, Hoskin *et al.* (2000b) found a correlation between pepsinogen levels and *Ostertagia*-type burdens, whilst others were not able to show a relationship with burdens (van der Heide 2009). Therefore, it is not surprising that even with higher burdens of *Osteragia*-type nematodes in this study no significant differences were found between groups, suggesting that this is not a good estimator of worm burdens in deer.

3.5.6 *Species Identification by PCR*

To be able to identify nematodes to species level in live animals, a collection of PCR protocols were used to estimate the prevalence of different GIN species in red deer in New Zealand (Chapter 4). The method used was largely adopted from that described by Bisset *et al.* (2014) for identifying common sheep and cattle GIN, but further protocols for some deer-specific GIN (Bisset unpublished) were also used.

In temperate climates, domestic farming species tend to host mixed nematode burdens. Because New Zealand is a temperate country and mixed grazing is a common farming system, an even wider range of nematodes are able to infect deer. The range of PCR reactions used to identify infective larvae (Chapter 4) encompassed 15 nematode species including common species in deer, sheep as well the more common species from cattle. Given the large number of nematode species, some primers had issues with cross-reactions. This was resolved by developing new primers, retesting some larvae and running the reactions in a specific order. The protocol followed did allow the identification of the most likely common species in deer with relatively high efficiency, but it does highlight that further work on developing and validating better primers without any cross-reaction between species found in deer needs to be undertaken before this approach can be more widely adopted.

3.5.7 *The effect of variations in metabolic age.*

As animals develop an immune response, despite them potentially receiving a constant challenge with parasites, some will be resilient and maintain their performance (Bisset *et al.* 2001) and others will be resistant to parasites having very low FEC but with lower weight gain. It is recognised that young ruminants proceed through three phases in the development of their immune response to GIN (Kimambo *et al.* 1988). The first is the hyporesponsive stage, the second the acquisition phase during which immunity is developing and the third is the mature expression phase. The impact of GIN during the first is minimal but is maximal during the acquisition phase. In contrast the maintenance of immunity during the expression phase will require the use of both protein and energy resources but usually doesn't result in overt disease. Greer and Hamie (2016) argue that the age at which various breeds of sheep reach the expression phase reflects the speed they attain metabolic maturity and they calculate this is 45% of their expected mature body weight. Comparing lines of sheep which develop lower faecal egg counts (resistant) with those with higher faecal egg counts (resilient) these authors argue the resistant animals have a lower metabolic age of maturity as reflected in these single trait selection lines. Since resilient animals exist in most groups of ruminants it provides an opportunity to reduce the number of anthelmintic treatments and only treat those who haven't performed. This is termed targeted selective anthelmintic treatment. Adopting this approach will allow farmers to reduce the number of treatments overall and also the selection pressure for anthelmintic resistance. This same concept is as likely to exist for deer as it does for sheep and probably reflects a higher susceptibility to nematodes in animals which have a higher mature body weight.

In the cross-grazing study (Chapter 6) three diagnostic methods were used as targets for selective treatment. These were faecal egg counts, faecal larval count and deer growth rate. For the last of these deer were treated if an individual deer's growth rate was less than 80% of the mean of the SP treatment group over the previous two weeks. In this study, the SP group was regularly treated and were intended to represent the maximum growth rate possible without any impact of parasites. For this experimental approach the use of liveweight was more sensitive than the triggers for FEC and FLC that had been adopted. The findings are similar to a study in sheep (Greer *et al.* 2009) which compared the live-weight gain against their predicted live weight gain using a predictive model. That model considered the efficiency of energy use together with environmental factors including the herbal quality and availability. In that model, if an animal doesn't achieved the weight gain expected, the individual animal was treated (Greer *et al.* 2009). The approach taken in the cross-grazing study (Chapter 6) required the use of a suppressive treated group (SP) as a benchmark to predict weight and was only meant for this study and it will not be feasibility to perform on farmed conditions. For use on commercial farms it will need to be modified to something similar to that described by Greer *et al.* (2009).

The cross-grazing study did indicate that weight was a more sensitive marker of parasite effects than FEC and FLC and this coincides with what has been found in other studies with red deer in New Zealand (Hoskin *et al.* 2000b; Hoskin *et al.* 2007).

3.6 Control

3.6.4 *Anthelmintics*

In New Zealand, there are only two action families of anthelmintics registered for deer, the benzimidazoles and the macrocyclic lactones. Of them, only benzimidazoles (albendazole, fenbendazole and oxfendazole) are licenced for used as an oral formulation in deer in New Zealand. In the cross-infection study (Tapia-Escárate *et al.* 2015b; Chapter 5) the deer were treated effectively with oxfendazole at double the recommended dose rate for deer (9.06mg/kg; Bomatak C®, Bayer New Zealand Ltd) together with oral abamectin (0.2 mg/kg; Combat AbaCare LV®, Virbac New Zealand Ltd) which is not registered for use in deer. In the cross-grazing study, the same combination was used, but the oxfendazole was used at the recommended dose (4.53 mg/kg; Bomatak C®, Bayer New Zealand Ltd) together with abamectin (0.2mg/kg). This combination was used principally to obtain high efficacy but have no residual activity to confuse the results of these studies. It is known that there is resistance to macrocyclic lactone anthelmintics in the *Ostertagia*-type nematodes in New Zealand (Lawrence 2011; Lawrence *et al.* 2012; Lawrence *et al.* 2013) including on the Massey

University Deer Unit (Hoskin *et al.* 2005) and on the Invermay Research Farm (Mackintosh *et al.* 2013). The combination used in the studies for this thesis were shown to be effective based on regular monitoring of faecal egg counts in many animals after treatment. Because Combat AbaCare LV is not licenced for deer in New Zealand, the tracer deer at AgResearch Invermay only received a double dose of oxfendazole (9 mg/kg; Oxfen C, Ancare NZ Ltd) when they entered the study and five weeks prior to slaughter to allow them to be slaughtered and their meat subsequently sold. In this location in 2012, three tracer deer, in the deer grazing with cattle group, had very high burdens of *Ostertagia*-type nematodes ($n \geq 40.000$) and the reason for this high number is uncertain but may involve anthelmintic resistance to the double dose of oxfendazole used to treat the tracer deer at this location at that time although it was not observed for the other tracer sets.

3.6.5 *Cross-grazing as an alternative control method*

The cross-grazing study (Chapter 6) demonstrated that for young deer after weaning cross-grazing with an alternative ruminant species offers some advantages over mono-grazing. Cross-grazing with cattle or sheep in the autumn was effective in reducing the lungworm burdens in weaner deer. This observation alone should prompt deer farmers to consider this control option.

For GIN, the results varied between the two options of either cross-grazing with sheep or cattle. *Ostertagia*-type nematodes of deer are considered to be the most important GIN so most focus in on their control. In the cross-grazing experiment, the higher burdens were found in the tracer deer in the DD group compared with the DS and the SP groups, indicating a reduction of the burdens of *Ostertagia*-type nematodes with alternating grazing with sheep. Even though the difference between the DD and DC groups was not significant, results showed a tendency toward lower counts in the DC groups compared to DD groups ($p=0.058$). Thus cross-grazing with either host is likely to be beneficial but didn't totally prevent infection with cross-grazing with sheep more effective in the present study.

Trichostrongylus spp. burdens were significantly higher in the tracers from DS groups and interestingly, the deer in the DS group received more anthelmintic treatments but still grew less well than either the DC or SP groups. This may suggest that abomasal *Trichostrongylus* spp. can be sufficiently important to cause effects on growth rates in farmed deer.

Taking all the parameters into account the DC group received fewer treatments than other groups and grew as well as the SP group, indicating that, within the constraints of this grazing study, cross-grazing with cattle was the most effective way to graze deer to obtain additional parasite control. Nevertheless, all three groups were still considered by the criteria used, predominantly weight gain, to need anthelmintic treatment.

The growth rate in the cross-grazing study (Chapter 6) was affected by the number of nematode larvae on the field and this by the differences in numbers of treatments given to each group. Nevertheless, even if the deer in the DS group received more anthelmintic treatments they still grew less well than the DC group that received fewer anthelmintic treatments. To reduce confounding factors the stocking rate of the alternative host species was adjusted every four weeks. However, the growth rates may also be affected by the grazing habits of deer that may be complemented by cattle whilst being competitive with the sheep (Hoskin 2007) and it was intended to keep pasture mass high enough to avoid this issue but the occurrence of very dry conditions in both years at Palmerston North required supplementary feeding which confounded the pasture availability. Deer and the alternating species (sheep or cattle) were separated by five paddocks in the rotation to provide the longest period possible between grazing and animals in all treatments were moved at the same time to the next paddock. Nevertheless, the need to give supplements did not prevent animals from becoming parasitised so the results were not compromised in terms of the determining the usefulness of cross-grazing or not.

3.7 Future perspectives

The studies in this thesis have highlighted a number of areas for future research.

The two pathogenicity studies (Chapter 2 and 3) did not achieve the aim of investigating subclinical effect of *Ostertagia*-type nematodes as both studies were confounded by the inclusion of *Oesophagostomum* spp. in particular, but also *Trichostrongylus* species.

Even a study where just *Ostertagia*-type nematodes were involved should really study each species separately.

Another missing piece in this thesis was to clearly establish the pathogenicity of the newly recorded *O. sikae*. Larvae of this nematode were obtained from Massey University-born weaner red deer and adult worms were identified from the deer infected with this larvae (Chapter 2). However, this nematode did not appear again in the large intestine of the tracers of the cross-grazing study (Chapter 6) that was run on the same farm. The results from the initial pathogenicity study suggested it might have been the cause of the nodule formation observed but there is some evidence that large infections with *O. venulosum* may also result in nodule formation. Thus there is a need to clarify the epidemiology and pathogenicity of this nematode and determining whether nodule formation may be found or is associated with the magnitude of the larval dose and/or due to concurrent infections with other *Oesophagostomum* species.

Future studies should continue to explore further development of diagnostic tools for monitoring GIN in deer. For example, in the cross-grazing study weight gain was almost exclusively the trigger for drenching animals and not faecal egg counts. In the pathogenicity study, pepsinogen values did not provide a useful correlation with the burden of *Ostertagia*-type parasites. An overarching meta-analysis on the usefulness of egg output and worm burden in young and adult deer would be a useful next step. Some studies have demonstrated that in adult deer egg output doesn't correlate with worm burden and thus FECs are not useful whilst in young deer the information is variable.

The development of the PCRs used in Chapter 4 were critical to allow that study to be conducted and it identified the prevalence of a wide range of species of nematodes in deer. There were still a number of samples remaining that were not identified possibly suggesting different species were present. Further work is required to develop and validate better primers without cross-reaction between nematodes species of deer, cattle and sheep. If these primers were available there is a potential role for qPCR with faeces to be used to provide a quantification of GIN within an animal.

3.8 Conclusion

This thesis supports the evidence that deer nematodes in the sub-family Ostertagiinae (=*Ostertagia*-type; *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera* and *Ostertagia leptospicularis*) are the most important GIN in deer. The cross-infection of nematodes from sheep into deer indicated that some species including *T. axei, O. venulosum* and *H. contortus* have the potential, if permitted by the environmental conditions, of building up and creating a clinical or a production problem for deer as a host. In addition, cross-grazing with cattle, in particular, was effective in reducing the effects of GIN burdens in the young deer, more so than cross-grazing with sheep. Cross-grazing with either cattle or sheep in the autumn was effective in reducing the lungworm numbers in weaner deer. Overall, even if cross-grazing with sheep or cattle did not provide sufficient control to completely avoid use of anthelmintic treatments, it did reduce the number of treatments required. Given the recent development of anthelmintic resistance in GIN of deer in New Zealand other control options need to be developed to reduce this dependence on use of anthelmintics.

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Appendices

Appendix 1 Supplementary information for Chapter 1. Literature review.

1.1 Revision of scientific publication of cross- and co-grazing with ruminants

Cross= The animals in th study were cross-grazing, Co-= the animals in the study were co-grazing, FEC= Faecal egg counts, WC= Worm counts, L3= Infective larvae, Yrs= Years, M= Month, Wk= Weeks, X= per, Alter= Alternate, NS= Not significant, S= Significant, diff= Differences, Dec= December, Jan= January, Feb= February, Jul= July, Oct= October, Rye= Ryegrass, Whc= White Clover, anim/ha= Animals per hectare, LW= live weight, LWG= liveweight gain, O. ost= *Ostertagia ostertagi*, O. circ= *Ostertagia circumcincta*, H. cont= *Haemonchus contortus*, H. placei= *Haemonchus placei*, T. col= *Trichostrongylus colubriformis*, Nemat= *Nematodirus*, C. onc= *Cooperia oncophora*, C. punct= *Cooperia punctata*, C. spatula= *Cooperia spatulata*.

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
1	Pregrazed by heifers → (**1**)undosed cattle "control" (**2**)grazed by sheep for 2 M (**3**)by sheep for 4 M (**4**)by sheep for 6 M (**5**)spelled for 4 M (**6**)cattle dosed 2 weekly X 4 M. Then regrazed by cattle for 2, 6, 8, 10 and 12 M - 1 steer killed at end of each period for WC and by 3 consecutive sets of tracer (n=?) at 6-8, 8-10, 10-12 M after grazing	Australia/ Barger and Southcott/ 1975	**sheep/cattle** 5 steer calves in group (1) (4) (6) and 4 calves on group (2) (3) (5)	1	Treatment and test animals "yearlings". Tracers = 4-8 M.	→group (6) fortnightly →ewes and steer calves before entering.	yes	no	**FEC**: **NS diff**	Steer and Tracer WC. Only qualitative comments, no statistical comparison. The major treatment effect were on *O. ost.* *O. ost* [(2), (3), (4), (6)] < (1). *T. axei* [(2), (3), (4), (5), (6)] < (1) *C. oncophora* (6) < (1)(2)(3)(4)(5) but peak in Dec in (1) & (5) after that very low number	In pasture in (1) the highest number of L3 of all spp.

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
2	**Pregrazed by sheep** →**(1)**6wk cattle; **(2)**6wk sheep; **(3)**12wk cattle; **(4)**12wk sheep; **(5)**24wk cattle; **(6)**24wk sheep. **Pregrazed by cattle** →**(7)**6wk cattle; **(8)**6wk sheep; **(9)**12wk cattle; **(10)**12wk sheep; **(11)**24wk cattle; **(12)**24wk sheep. Tracers grazing X 1 M. If pregrazed by sheep: 10 tracer lambs if pregrazed by cattle: 3 tracer calves	Australia/ Southcott and Barger/ 1975	**sheep/cattle** per treatment =3 steers / 18 ewes, tracers=10 sheep and 3 calves (X 1 M)	2	Treatments=15 M. Tracers= 5, 6.5, 9 M.	→Tracers sheep treat fortnighly untill weaning and then weekly, until requiered. →Tracer calves treat fortnighly post weaning. Treatment group: before entering	yes	no	No counts	→ **Lambs** : H. cont (1)(3)(5) < (2)(4)(6) *O.circ* (3)(5) < (4)(6) *T.col*(1)(3)(5) < (2)(4)(6) Nemat (5) < (6). →**Calves** C.onc (10) < (9)(12) < (11) O.ost (10)(12) < (9)(11) However *H.cont* (5)(11) < (6) (12) some calves (+) to *T.col*.	
3	**Slighty pregrazed by sheep** → **(1)**sheep-lamb (4.9 anim/ha) **(2)**sheep-lamb (7.4anim/ha) **(3)**sheep-lamb (9.9/ha) **(4)**sheep30%+steers70% **(5)**sheep40%+steers60% **(6)**sheep50%+steers50% **(7)**sheep70%+steers30%. For worm burden two sheep slaughter (heavier & lighter) X treat Two replications of each grazing treatment.	Australia/ Arundel and Hamilton/ 1975	**sheep/cattle**	1	Treat ewes=15M Steers= 7 M. Tracers lambs= 4M	→Ewes and Steer, at start Dec, Lambing May, end-winter late Aug. →Tracer Lambs, late May →lambs, 6 july, 2 august, 23 august	no	yes	**LWG=NS**	→Different stocking rates NS results. →Cograzing vs single grazing O. circ (1)(2)(3) > (4)(5)(6)(7). T. axei [(4)(5)(6)(7)]> [(1)(2)(3)] C. onc only present in (5)(6)(7).	

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
4	**Pregrazed evenly by sheep and cattle** → **(1)** SC sheep & cattle not alternate **(2)** SC6 sheep & cattle alternate every 6M **(3)** SC12 sheep & cattle alternate every 12M Over 3 years replicated at 2 sites. Trial on post weaning management.	Australia/ Barger and Southcott/ 1978	**sheep/cattle** site1= 24wethers or 4steers site2= 18whethers or 3steers. X group.	2	young sheep and cattle	2 treats per year →sheep at weaning and 6M later →Cattle when arrived to experiments and 6M later **→(3) In 3rd year, monthly**.	yes	no	**FEC** In sheep only **S** in April year1&2 (2)<(1)(2) year3 (2)(3) < (1) Not a pattern in cattle. **LW** **A**pril year1&2 **S** (2) > (1)(3) year3 **S** (2)(3) > (1)	In sheep (2) < (1) on *H. cont*, *T.col*, *T.axei* and *Nemat*. But (1) < (2) on *C. onc* (3) < (1) for all spp. except *H. cont* and *O. circ* NS but tendency to (1)> (2)(3).	Fleece weight year1&2 (2)>(1) Mortalities Year1&2 (2) < (1)(3) year3 (2)(3) < (1)
5	**(1)**sheep **(2)**cattle **(3)**mixed grazing **(4)**alternate grazing. The study was carried on for 5 years.	Norway/Helle/ 1981	**sheep/cattle** (1) 10ewes+20lambs (2) 12calves (3) 6calves & 5ewes+10lambs (4) 12calves & 10ewes+20lambs.	1, and 2 in the alternate.	calves 0.5-1 year old	→in (1)(3)(4) ewes before grazing & lambs end of summer. →only on 3rdyear calves in (2) twice →4&5year on calves in (2)(3)	yes	yes	**FEC** on calves (4)<(2)(3). **LW** in lambs > in (3) in calves > in (4)	Only cross-infection with *Nemat battus* other nematodes were not important for the cows.	
6	**(1)**S sheep in sheep pasture (may-sept) **(2)**K cattle in cattle pasture (may-sept) **(3)**KS/SK Alternate, cattle in cattle pasture / sheep in sheep pasture X 2M (may-july), & X 2M alternate (July-Sept)	Switzerland/ Inderbitzin *et al.*/ 1981	**sheep/cattle** **(**1)8-10ewes + 8-13lambs (2)6-8 cattle (3)6-8cattle / 8-10ewes+8-15lambs	5 in(1)(2) & 10 in(3) rotation every 30 days	cattle 5-6M at the start	in (1)(2)(3) only in the 4th year in summer	yes	no	**FEC** Year1and2 **NS** Year3 at the end (July-Sept) **S** (2) > (3) Year4 at the end (august-sept) **S** (2) > (3) **LWG= NS.**	**NS** between K and KS	
7	**S/S** only sheep**. S/C** altern sheep /cattle **(1)**S/S dec drench **(2)**S/S dec/feb drench **(3)**S/S dec/feb/july drench **(4)**S/S two weekly **(5)**S/C dec drench **(6)**S/C dec/feb drench **(7)**S/C dec/feb/july drench **(8)**S/C two	Australia/Donald *et al*./ 1987	**sheep/cattle** sheep= 23/ha. Cattle= put and take depend on pasture.	S/S=1 S/C=2 but in year2 (3) subdivided in 3 for rotation.	weaner sheep and young cattle	Cattle undosed. Sheep depend on groups, but in year2 (2)&(3) change to 5 drenches over	yes	no	**FEC** not in. In sheep **LWG S** S/C > S/S. Sheep in (5) =(8)(4)	Burderns in sheep S/C < S/S for *Ostertagia* H. cont on summer & early autumn Trichostrongyluys over the year.	Fleece weight **S** S/C > S/S. Sheep in (5)= (8)(4)

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
	weekly. Study over 3 years but only 2 years considered (1st year drought)					summer.				but (8)=(4) Within S/C **NS**	
8	**(1)**ewe+lamb **(2)**ewe+lambs/cow+calves **(3)**cow+calves. 4 lambs and 3 calves were kill for worm burdens. Study over 3 year.	US/ Jordan *et al.*/ 1988	**sheep/cattle** (1) 30ewes+lambs (2) 15ewes+lambs/ 3cows+calves (3) 6cows+calves	3 (3 rep, not rotation)	cow+calves & ewes+lambs	Adults every year before entering. Offspring only 2nd & 3rd year before entering.	no	yes	**FEC** monthly (results not in). **LW NS**, but lambs in (2) > (1) calves in (2) < (3)	**NS,** but lambs (2) < (1) calves (2) > (3)	
9	Pregrazed by cattle → **(1)**calves **(2)**calves/sheep **(3)**sheep/calves. Pregrazed by ewes and lambs → **4)**sheep. Study over 4 years.	UK/ Bairden *et a.l*/ 1995	**sheep/cattle.** X paddock 6calves or 6ewes&twin lamb	1	calves ewes and lambs	ewes at turnout.	yes	no	**FEC** **NS** between treats.	on calves **S** in *O. ost* in year2 and on *C. onc* in year1.	Supplementation when needed.
10	**Pre-grazed by sheep then 58 days spell →(1)** cattle **(2)** cattle/sheep **(3)**sheep. Study over 1 year	Brazil/ Amarante *et al.*/ 1997	**sheep/cattle** (1) 5steer (2) 5steer/ 16ewes (3) 16ewes	1	Ewes >2 yrs steers 8 M	1 steer of group 2 two treatments in may and july. And from dec-april tick control. Sheep treated 3 M after start and when high fec or clinical signs of parasitism. Tracer =25 days before enter.	no	yes	No statistics on it.	Burdens on tracer lamb= every M in paddocks and tracer calves only at the 8th M of the trial. Also 1 ewe and 1 steer (> fec) from 2). *H. placei* and *C. punct* more adapted to cattle, *T. axei* and *C. spatul* apparently more adapted to cattle, *T. col* and *H. cont* more adapted to sheep, *C. curt* apparently more adapted to sheep.	3 died in group (2) and 4 died in group (3)

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
11	**clean pasture (recently sown)** → **(1)**sheep only (Rye/whc 100%) (**2**)sheep/cattle 1 grazing (Rye/whc 100%) (**3**)sheep/cattle 2 grazing (Rye/whc 100%) (**4**)sheep/cattle 3 grazing (Rye/whc 100%) (**5**)sheep/cattle 4 grazing (Rye/whc 100%) (**6**)sheep only (lucerne30% and rye/whc 70%) (**7**)sheep only (lucerne30% and mix 70%) (**8**)sheep/cattle 2 grazing (lucerne30% and mix 70%). Rotation in all groups every 28 days in spring and every 98 days in winter.	NZ/ Moss *et al.*/ 1998	**sheep/cattle** All groups had 30ewes_39lambs plus (2)(3)(4)(5)(8) had 18cattle.	8 farmlet subdivided in 3-10 paddocks (depend on season)	cattle 7 M replacement	Ewes not treated, only before add in. Lamb drench twice 21 Jan and 15 March (fec>1100). Cattle treated every 3 weeks.	yes	no	**NS** diff found for all treatments.	**Burden on 4 females and 4 males at begging (6m) and end of autumn (8m), each year.** NS diff found for all treatments.	
12	Pre-grazed as the treatments the preceding year → **(1)**SS=sheep only (May to Oct) (**2**)CS=cattle (May to Jul)sheep (to Oct) (**3**)CS+S= Cattle and sheep (May to Jul) and then sheep (to Oct) (**4**)CS+CS= cattle and sheep (May to Jul) and then cattle and sheep (to Oct).	UK/ Marley *et al.*/ 2006	**sheep/cattle** ewes lambs (ewe 1 : 1.4 lambs) cattle = yearlings (cattle1 : 4 ewes) post weaning= 24 lambs per treatment	1	Lambs, steers yearlings (born in spring)	lambs at weaning before entering and every 28 days	yes	yes	→FEC (1) Highest and (2) Lowest. →LWG (1) Lowest and (4) Highest	No counts	

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
13	**(1)**cattle and sheep interchange every 32 days **(2)**cattle and sheep interchange every 96 days **(3)**cattle and sheep interchange every 192 days. Tracer lambs (3 M) grazed for 32 days with sheep and then housed for 28 days. Also all the steers.	Brazil/ Rocha *et al.*/ 2008	**sheep/cattle** (1)(2)(3) 22sheep (66 total) & 4cattle (12 total) →tracer lamb 4 on each occasion.	16 (3 groups)= 48	→sheep 30 of 1 yr & 36 of 2-5 year →cattle 2 yr →tracer lamb 3 M	ewes end of gestation and when trigger FEC higher than 4000 or PVC lower than 21% was reached.	yes	no	**FEC** **NS** exceptions: March 2004, (3) > (2) August 2005, (1) > [(2) (3)] (P < 0.05).	No control with one sp. **NS** diff between diff times. In tracer lambs mainly *H. cont* & *T. col.* In cattle mainly, *H. sim*, *C. punc* & *O. rad.* **NS** cross-infection.	→ pasture plus feeding. →Culture larvae. % of *Haemonchus* in cattle (3) > (1)(2) and *Cooperia* (1) > (2)(3)
14	→**1st stage (1)**supressed wethers grazing 2 cycle of 21 days (**2**)continuous sheep "CS" (**3**)continuous cattle "CC" [(1)(2)(3) rep in 3 paddocks] →**2nd stage** bearing ewes to test 1st stage. Trial for preparing pasture for spring lambing. Tracers, first in holding paddock (HP) for +100 days, then for 13 days in pens (PEN). Finally tracers grazed for 14 days. At pre 1st stage and pre and post 2nd stage.	Australia/ Bailey *et al.*/ 2009	**sheep/cattle** →1st stage (1)45wethers X 3 (2)13wethers X 3 (3)2steers X 3 →2nd stage 10pregnant_ewes & 10lambs born (X paddock) →Tracer_sheep 2 per group each time.	3 (total 9 of 2 ha)	→1st stage (1)(2) 28 M (3) 18 M →2nd stage ewes and lambs →Tracer sheep 16 M	→1st stage and 2nd stage on arrival. In (1) twice. In (2) when trigger reached. →in tracers. HP, bolus (every 100 days) after that, weekly. PEN, 3 treat in 3 days consecutive diff combinations.	yes	no	**FEC S** [(1) (3)] < (2). **LWG** ewes and lambs at weaning [(1) (3)] > (2).	% of reduction of burdens →from beginning 1st stage-beginning of 2nd stage in (1)97.7% > (3)96.9% > (2)88.5% →from beginning 1st stage-end of 2nd stage (1)87.9% > (3)85.6% > (2)26%	in 2nd stage barley was distributed twice per week. Ewes grazing in (1) 4% more clean wool than in (2).
15	(**1)**Alternate heifers and ewes+lambs (**2) Control** heifers (**3)C**ontrol ewes+lambs. Study during Dry, Intermediate, Rainy season. 5 lambs born on the study per treatment (4 of the 5 lambing period), were used for worm burdens.	France "Martinique"/ Mahieu and Aumont/ 2009	**sheep/cattle** **(**1) 60 ewes & 14 heifers 2) 11 heifers (3) 60 ewes	1)=8 2)=4 3)=5	heifers (200kg). Ewes 1-8yrs & replacement 7-11 M.	→Lambing ewes twice (5wks after lambing and at weaning). →Lambs twice (5wks before weaning and at weaning). No treat on heifers or pregnant ewes.	yes	no	**FEC** →In young lambs <40days (3) > (1) in dry & rainy seasons. →in older lambs (1)<(3), except at weaning in the rainy season. →In lactating ewes treat effect only in intermediate season. →NS in heifers	Burdens →on lamb. *Haemonchus* S (3)>(1) *Cooperia* S (1)>(3). NS for *Trichostrongylus*. →on Heifers. In (1) suggestion of infection with *H. cont*	Measurements of PCV in ewes and lambs (3) < (1)

	Treatments	Country/ Author/Year	Species/Number of animals	Paddocks per group	Age of animals	Anthelmintic treatment	Cross-	Co-	FEC/LW	Worm burdens	Other
16	Clean pasture (recently sown)→ (**1)**Cow+calves (**2)**Cow+calves & lambs	Finland/ Sormunen-Cristian *et al.*/2012	**sheep/cattle** (1)cow+calves 8 (2)cow+calves 8 + lambs 45.	3	year 1 calves=46d lambs=81d year 2 calves=66d lambs=91d	No treatment.	no	yes	**FEC** Eimeria (1) > (2) in June 2003 and August 2004. For Strongylids S diff between treatments in 2004.	No counts	Supplementation when needed.
17	pregrazed by goats→ Different stocking (**1)**100% goat "G" (**2)**75%G (**3)**50%G (**4)**25%G and heifers to complete the %. six paddocks and weekly rotations therefore 4 weeks of rest for every paddock.Rep 3 per year a total of 10 rep. The trial last for 4 years.	France "Guadeloupe"/ Mahieu/ 2013	**goat/cattle** 15-17 kids and 5-8 heifer per group X 4 years	(1)=5 others =6	kids=4-7 m. Weaned heifer	kids=weaning and 5 M	yes	no	**FEC= S (4)**25%G < (1)100%Cntrl. **LWG**= (4)25%G > (3)50%G (2)75%G (1)Cntrl. **LW** = Growth rate increased 59% in (4)25%G and 34% in (3)50%G.	No counts	Death rate were lower in 4)25%G and 3)50%G than 1).

Appendix 2 Supplementary information for Chapter 2. Analysis of the pathogenicity and diagnosis of GI nematode infections in young farmed deer – Initial Study

2.1 Data- Blood parameters

Treat= Treatment Group, LD= Low dose, MD= Medium dose, HD= High dose, Pen= allocation of the deer on the pen, Anim= Identification number of the deer, Day= time before (-14) and after (1-36) beginning of trickle infection, Alb= Albumin (g/l), TP= Total protein, Glob= Globulin (g/l), Agr= Albumin to globulin ratio, Wbc= White blood cell count (x 10^9 cells/l), Rbc= Red blood cell count (x 10^9 cells/l), Hgb= Haemoglobin (g/l), Mcv= Mean corpuscular volume (l/cell), Mch= Mean corpuscular haemoglobin (g/cell), Neut= Neutrophils (x10^9 cells/l), Lymph= Lymphocytes (x10^9 cells/l), Mono= Monocytes (x10^9 cells/l), Eosi= Eosinophils (x10^9 cell/l), Baso= Basophils (x10^9 cell/l), Luc= Large unstained cells, Pep= Pepsinogen (iU).

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
Control	7	1	-14	29	55	26	1.12	4.88	12.14	148	30.5	12.2	26.3	61.2	9.5	0.3	2.4	0.3	.
Control	8	3	-14	35	59	24	1.46	5.65	11.45	151	31.7	13.2	30.3	62.5	3.8	0.2	3	0.2	.
Control	7	10	-14	33	63	30	1.1	6.03	11.59	163	34.3	14	34	57.7	5.8	0.2	0.9	1.4	.
Control	8	11	-14	32	64	32	1	5.53	11.64	158	35	13.6	35.3	59.7	1.6	0.4	2.6	0.4	.
Control	7	14	-14	34	67	33	1.03	6.45	10.76	134	30.8	12.4	30.1	63.2	3.9	0.2	1.9	0.7	.
Control	7	15	-14	35	61	26	1.35	6.14	13.33	170	32	12.8	19.1	75.1	3.2	0.2	1.8	0.5	.
Control	8	18	-14	33	63	30	1.1	7.93	11.58	150	32.5	12.9	37.8	56.1	2.6	0.2	2.8	0.5	.
Control	8	21	-14	35	60	25	1.4	8.68	11.9	160	33.7	13.4	39.2	55.2	3.8	0.2	1.3	0.3	.
Control	7	24	-14	32	58	26	1.23	10.9	10.92	143	33.1	13.1	33.1	57	6.5	0.1	2.8	0.4	.

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
Control	8	26	-14	33	60	27	1.22	8.13	12.69	156	30.7	12.3	29.1	64.3	3.8	0.7	1.7	0.5	.
Control	7	47	-14	38	63	25	1.52	3.91	11.84	188	38.9	15.8	10.9	75.5	10.8	0.2	2	0.6	.
Control	8	61	-14	40	68	28	1.43	5.99	10.48	158	37.3	15.1	36.7	52.1	8.3	0.1	2.5	0.3	.
LD	5	5	-14	32	58	26	1.23	6.5	10.23	144	34.7	14.1	25	69.8	2.6	0.1	1.8	0.7	.
LD	5	6	-14	29	57	28	1.04	11.08	9.42	128	33	13.6	29.1	61.5	7	0.1	1.6	0.6	.
LD	5	13	-14	34	66	32	1.06	7.68	10.93	136	30.3	12.5	36.8	55.2	4.7	0.1	2.8	0.3	.
LD	5	20	-14	37	66	29	1.28	7.43	11.73	146	31.5	12.5	30.6	62.5	3.8	0.1	2.7	0.3	.
LD	6	22	-14	34	64	30	1.13	9.35	11.53	139	30.5	12.1	37.2	53.6	7.1	0.1	1.6	0.3	.
LD	6	23	-14	37	63	26	1.42	6.37	9.71	112	28.3	11.5	21.8	71.5	3.3	0.2	2.6	0.6	.
LD	5	38	-14	37	70	33	1.12	7.48	13.36	160	29.9	12	29.2	64.3	3.5	0.3	2.4	0.3	.
LD	6	53	-14	35	65	30	1.17	6.36	11.57	159	35.2	13.8	19.8	74.5	1.8	0.2	3.3	0.4	.
LD	6	56	-14	34	61	27	1.26	8.39	13	171	32.7	13.2	49.1	44.6	3.9	0.1	1.5	0.7	.
LD	6	68	-14	37	70	33	1.12	6.6	12.11	161	34.1	13.3	25.3	63.6	5.7	0.1	4.6	0.5	.
LD	5	104	-14	31	60	29	1.07	9.15	13.43	175	32	13	26.7	65.8	4.9	0.9	1.2	0.6	.
MD	4	4	-14	33	65	32	1.03	6.05	11.98	145	29.9	12.1	26.9	67.6	3.1	0.1	1.8	0.4	.
MD	2	8	-14	32	62	30	1.07	6.64	12.24	151	30.4	12.3	30.5	64.8	2.5	0.3	1.8	0.2	.
MD	4	16	-14	35	66	31	1.13	6.41	13.27	167	31	12.6	31	64.1	2.9	0.1	1.6	0.3	.
MD	4	25	-14	31	62	31	1	5.6	10.74	141	34.2	13.1	28.3	66.4	3	0.1	1.8	0.3	.
MD	4	28	-14	36	68	32	1.13	7.17	14.08	168	29.3	11.9	26.2	67.8	3.4	0.2	2	0.3	.
MD	2	29	-14	33	64	31	1.06	7.78	11.84	141	31.2	11.9	36.7	57	3.9	0.1	1.6	0.8	.
MD	4	39	-14	32	64	32	1	6.89	12.5	185	36.5	14.8	22.3	69.6	4	0.3	3.3	0.5	.
MD	2	78	-14	39	71	32	1.22	4.79	12.65	164	32.1	12.9	31.6	58.3	6.2	0.4	3.1	0.4	.
MD	4	81	-14	33	62	29	1.14	5.22	11.82	157	33.2	13.3	19.8	66.5	8.8	0.2	4.2	0.6	.
MD	2	88	-14	34	63	29	1.17	5.76	11.26	144	32.1	12.8	42.3	50.8	3.7	0.2	2.7	0.3	.
MD	2	98	-14	31	64	33	0.94	6.76	10.7	146	33.8	13.6	32.7	56.3	7.1	0.2	3.1	0.7	.
HD	1	2	-14	39	68	29	1.34	8.69	12.2	174	34.4	14.3	28.1	64.1	3.5	0.1	3.9	0.2	.
HD	3	7	-14	35	62	27	1.3	9.02	12.81	177	34.1	13.8	20.6	73.3	3.7	0.2	1.9	0.3	.
HD	1	9	-14	33	60	27	1.22	9.17	11.96	155	32.4	13	41.7	52.8	2.6	0.1	2.1	0.7	.
HD	1	12	-14	32	66	34	0.94	4.6	11.17	150	33.8	13.5	29.7	64.4	4	0.1	1.6	0.2	.

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
HD	1	17	-14	36	65	29	1.24	4.78	12.63	156	30.9	12.3	35.3	59.7	2.6	0.2	2	0.1	.
HD	3	19	-14	37	66	29	1.28	12.51	10.76	135	31.6	12.6	28.2	62.6	4.2	0.1	4.5	0.4	.
HD	3	27	-14	34	60	26	1.31	8.76	12.59	153	31	12.2	21.5	71.2	4.5	0.1	2	0.8	.
HD	3	30	-14	32	65	33	0.97	6.61	11.7	145	30.7	12.4	27.6	64.7	4.4	0.1	2.4	0.9	.
HD	1	42	-14	35	65	30	1.17	10.56	12.63	151	30.2	12	44.5	50.1	3.4	0.3	1.2	0.5	.
HD	3	92	-14	29	57	28	1.04	5.45	13.13	159	29.4	12.1	19.7	73.7	4.1	0.1	1.6	0.8	.
HD	3	115	-14	33	63	30	1.1	5.68	10.64	152	36.9	14.3	24.3	68.9	4.9	0.1	1.3	0.4	.
Control	7	1	1	30	58	28	1.07	4.95	12.6	150	30	11.9	30.2	63.7	3.7	0.7	1.3	0.4	-7.4
Control	8	3	1	33	55	22	1.5	6.2	10.55	140	31.2	13.2	37.9	56.5	2.4	0.2	2.6	0.3	-2.8
Control	7	10	1	35	66	31	1.13	5.06	10.96	161	33.8	14.7	26.8	67.1	3.3	0.7	1.1	1	-2.1
Control	8	11	1	33	63	30	1.1	6.12	10.73	149	33.9	13.9	35.8	58.3	3	0.3	2.4	0.3	-2.9
Control	7	14	1	35	72	37	0.95	6.63	10.79	135	30.5	12.5	36.4	57.9	2.7	0.3	2.1	0.6	-1.4
Control	7	15	1	36	63	27	1.33	7.91	11.93	156	31.2	13.2	24.7	71.6	1.5	0.2	1.7	0.3	-1.5
Control	8	18	1	35	65	30	1.17	7.45	11.45	154	31.9	13.4	35.7	60.4	1.3	0.1	2.4	0.2	-0.7
Control	8	21	1	35	62	27	1.3	9.17	11.23	153	32.8	13.7	34.8	60.1	3.2	0.3	1.5	0.2	-1.7
Control	7	24	1	33	61	28	1.18	12.11	10.64	142	32.2	13.4	30.1	63.7	3.3	0.2	2.4	0.3	2.6
Control	8	26	1	34	60	26	1.31	8.51	12.03	151	30	12.6	34.6	60.1	3.4	0.2	1.4	0.4	-2.1
Control	7	47	1	34	60	26	1.31	4.46	10.99	177	38.1	16.2	12.3	77.1	7.2	0.3	2.1	1	-4.5
Control	8	61	1	40	71	31	1.29	5.58	9.61	147	36.4	15.3	35	57.3	5.1	0.2	2.2	0.2	-1.3
LD	5	5	1	31	59	28	1.11	8.21	11.02	154	33.7	13.9	29.7	66.2	2.1	0.6	1.1	0.3	-3.4
LD	5	6	1	30	61	31	0.97	11.96	8.92	122	32.9	13.6	37.8	56.4	3.8	0.5	1.1	0.4	-11.4
LD	5	13	1	35	67	32	1.09	8.43	9.73	126	29.7	12.9	47	47.1	4.3	0.3	1.1	0.2	-1.0
LD	5	20	1	41	69	28	1.46	9.14	13.02	169	31.6	13	38.7	54.4	4.2	0.8	1.5	0.4	1.6
LD	6	22	1	35	64	29	1.21	11.04	10.94	137	30	12.5	32.8	61	4.6	0.2	1.2	0.2	-1.2
LD	6	23	1	37	63	26	1.42	5.87	9.95	115	27.5	11.6	26.2	66	4.9	0.8	1.6	0.5	-1.0
LD	5	38	1	37	70	33	1.12	7.7	12.74	155	29.1	12.2	41.3	52.8	3.7	0.7	1.1	0.3	-1.2
LD	6	53	1	35	65	30	1.17	7.19	10.47	148	34.7	14.2	35.3	59.6	2.5	0.6	1.7	0.3	-1.2
LD	6	56	1	35	66	31	1.13	6.48	11.18	154	32.6	13.8	46.5	46.9	4.7	0.4	1	0.5	0.5
LD	6	68	1	38	71	33	1.15	7.32	12.34	165	33.2	13.4	33.5	59.1	4	1.3	1.8	0.2	0.7

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
LD	5	104	1	32	62	30	1.07	7.36	12.8	161	31	12.6	40.3	55.4	2.2	0.9	0.6	0.6	-0.4
MD	4	4	1	30	60	30	1	5.23	12.1	148	29.4	12.2	32.3	62.7	2.4	0.8	1.1	0.6	-3.4
MD	2	8	1	35	67	32	1.09	7.39	12.13	154	30.8	12.7	40	55.5	2	0.6	1.7	0.3	-4.7
MD	4	16	1	38	68	30	1.27	6.55	12.98	163	30.8	12.5	42.3	52.6	3.2	0.5	1.2	0.2	-3.9
MD	4	25	1	32	60	28	1.14	5.97	11.14	150	33.9	13.4	40.6	54.4	3.4	0.4	0.7	0.6	-0.3
MD	4	28	1	37	68	31	1.19	7	12.47	149	28.5	12	36.1	58.3	3.6	0.6	1	0.5	-0.5
MD	2	29	1	33	61	28	1.18	6.95	12.69	153	31	12	53.6	36	7.3	1.2	1	0.9	-3.6
MD	4	39	1	33	65	32	1.03	6.65	12.39	188	36.3	15.2	36.9	55.4	5.3	0.8	1.2	0.3	0.0
MD	2	78	1	39	71	32	1.22	5.93	12.7	167	31.6	13.2	41.9	52.5	3.3	0.8	1.3	0.2	-0.7
MD	4	81	1	31	58	27	1.15	6.33	10.62	143	32.2	13.5	53.6	43.7	0.6	1	0.8	0.3	1.0
MD	2	88	1	32	62	30	1.07	7.79	10.44	138	31.6	13.2	68.3	26.9	2.8	1	0.7	0.3	-2.0
MD	2	98	1	29	60	31	0.94	6.52	10.21	143	33.1	14	48.2	45.4	4	1.1	0.7	0.7	-0.5
HD	1	2	1	37	65	28	1.32	7.68	8.91	126	33.3	14.1	39.7	54.3	3.6	0.4	1.9	0.2	-4.2
HD	3	7	1	31	59	28	1.11	8.01	10.12	141	33.7	13.9	39.5	55.2	3	1	0.8	0.4	-7.0
HD	1	9	1	33	62	29	1.14	13.55	11.73	157	31.9	13.4	61.7	32.5	2.7	0.6	1.7	0.8	-4.2
HD	1	12	1	32	64	32	1	4.45	11.02	149	32.9	13.6	44.2	51	2.6	0.7	1.3	0.3	-1.7
HD	1	17	1	35	63	28	1.25	5.83	12.52	158	30.6	12.6	53.6	40.8	4.1	0.6	0.6	0.4	0.5
HD	3	19	1	34	63	29	1.17	10.68	10.62	136	31	12.8	43.7	52.2	1.6	0.6	1.4	0.3	0.5
HD	3	27	1	32	57	25	1.28	8.54	12.09	152	29.9	12.5	42.7	51.2	3.4	0.4	1.1	1.2	-4.4
HD	3	30	1	33	65	32	1.03	6.55	11.53	147	30.3	12.7	42.1	50.1	5.1	0.5	1	1.2	-3.6
HD	1	42	1	36	66	30	1.2	8.18	12.11	148	29.8	12.3	61.6	31.2	5.1	1.2	0.6	0.3	-2.8
HD	3	92	1	25	52	27	0.93	8.46	10.77	132	28.8	12.2	60.8	34.2	2.9	0.9	0.4	0.7	-0.4
HD	3	115	1	30	64	34	0.88	7.14	10.29	150	36.1	14.6	51.6	42.8	3.7	0.8	0.8	0.3	-4.1
Control	7	1	8	29	58	29	1	7.03	11.82	139	29.7	11.8	35.7	57.7	4.4	0.7	1.4	0.1	2.6
Control	8	3	8	35	60	25	1.4	7.09	13.25	167	31.5	12.6	34.5	60.2	1.9	0.4	2.8	0.2	1.1
Control	7	10	8	35	70	35	1	6.94	11.45	157	33.7	13.7	33.3	60.5	4.3	0.1	1.2	0.6	4.5
Control	8	11	8	34	64	30	1.13	7.33	12.3	164	33.6	13.3	28.3	67.1	1.7	0.2	2.5	0.2	0.4
Control	7	14	8	35	73	38	0.92	7.5	11.33	135	30.3	11.9	34.7	58.8	3.4	0.4	2.5	0.3	2.3
Control	7	15	8	35	65	30	1.17	7.5	12.84	161	31.4	12.5	15.9	77.8	3.7	0.2	2	0.4	0.1

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
Control	8	18	8	35	68	33	1.06	8.13	11.7	146	31.5	12.5	30.4	61.1	5.7	0.2	2.3	0.2	3.4
Control	8	21	8	35	65	30	1.17	11.39	12.07	159	32.2	13.2	36.4	59	2.9	0.2	1.3	0.2	1.3
Control	7	24	8	32	62	30	1.07	11.45	10.43	132	32	12.6	29.5	65.2	2.2	0.2	2.5	0.3	-5.8
Control	8	26	8	34	62	28	1.21	9.14	12.44	149	29.7	11.9	28.9	66.7	2.7	0.1	1.4	0.2	0.6
Control	7	47	8	32	60	28	1.14	4.85	11.37	174	37.5	15.3	10.9	80.9	5.3	0.2	2.1	0.5	4.0
Control	8	61	8	41	76	35	1.17	6.82	11.83	172	36.4	14.6	42.2	51.3	4.4	0.2	1.7	0.3	-0.4
LD	5	5	8	31	64	33	0.94	7.16	12.77	173	33.5	13.5	31.6	61.4	2.6	1.5	2.6	0.3	-2.3
LD	5	6	8	27	60	33	0.82	10.35	11.79	150	32.8	12.8	28	66.8	1.3	1.5	2.2	0.2	-0.5
LD	5	13	8	34	70	36	0.94	7.69	11.56	138	29.6	11.9	45.1	47.7	3.4	1.2	2.3	0.3	-6.2
LD	5	20	8	36	65	29	1.24	7.54	13.27	167	31.6	12.5	37.9	55.1	3.6	0.4	2.7	0.3	5.6
LD	6	22	8	35	67	32	1.09	11.99	14.02	169	30	12.1	40.5	50.4	5.3	0.7	2.9	0.2	2.1
LD	6	23	8	38	65	27	1.41	3.96	11.32	126	27.4	11.2	25.4	67.5	2.2	2.6	1.7	0.5	-1.7
LD	5	38	8	35	74	39	0.9	7.01	13.69	163	29	11.9	33.7	56.7	3	4.1	2	0.4	-16.1
LD	6	53	8	32	67	35	0.91	7.01	12.07	167	35.3	13.9	25.4	61.9	3	5.9	3.5	0.3	-1.8
LD	6	56	8	34	67	33	1.03	7.71	13.39	175	32.3	13.1	43.7	45.6	5.8	1.6	3	0.4	2.4
LD	6	68	8	33	73	40	0.83	6.22	11.9	152	32.7	12.8	26.9	60.5	4	4.4	3.7	0.5	0.3
LD	5	104	8	32	69	37	0.86	6.22	14.5	182	30.6	12.6	31.3	60	3.6	3	1.6	0.5	-1.0
MD	4	4	8	30	71	41	0.73	3.82	15.45	181	29.1	11.7	34	52.4	5.1	5.3	2.5	0.6	1.3
MD	2	8	8	32	67	35	0.91	7.31	13.54	161	30.3	11.9	43	48.6	2.7	1.3	4	0.4	-0.5
MD	4	16	8	35	70	35	1	9.06	11.7	144	30.5	12.3	56	38	3.4	0.8	1.2	0.6	-0.4
MD	4	25	8	31	64	33	0.94	6.8	12.12	159	33.6	13.1	28.2	64.1	4.4	1.5	1.5	0.4	4.5
MD	4	28	8	30	64	34	0.88	6.36	11.84	156	33.9	13.2	31.6	61.1	3.7	1.4	1.3	0.8	-2.2
MD	2	29	8	30	68	38	0.79	6.86	13.81	167	31	12.1	43	42.7	3.9	8.1	1.8	0.5	2.0
MD	4	39	8	31	72	41	0.76	5.14	13.25	191	36	14.4	23.4	60.6	2.5	10.8	2.4	0.4	0.0
MD	2	78	8	37	76	39	0.95	4.64	13.83	175	31.4	12.6	28.4	62.2	3.2	3.6	2.1	0.5	-3.5
MD	4	81	8	31	71	40	0.78	4.86	13.33	171	32.1	12.8	22.5	64	5.3	3.1	4.7	0.4	-1.4
MD	2	88	8	30	67	37	0.81	5.24	11.24	140	31.4	12.5	40.2	46	4	6.7	2.5	0.5	-2.2
MD	2	98	8	28	67	39	0.72	5.84	11.72	157	32.9	13.4	31.4	59.7	4.1	2.2	1.8	0.7	1.2
HD	1	2	8	37	73	36	1.03	6.08	12.12	163	33.3	13.5	32.1	58.7	4	4.6	6.9	0.5	3.2

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
HD	3	7	8	26	61	35	0.74	5.84	12.87	172	33.7	13.4	28	58.4	4.3	8.9	5.6	0.4	-2.1
HD	1	9	8	30	63	33	0.91	6.29	13.01	166	31.7	12.7	51.1	43.3	2.5	2.7	5.4	0.4	-4.8
HD	1	12	8	28	70	42	0.67	2.73	11.51	151	32.7	13.1	36.6	51.8	4.3	3.1	3.6	0.6	0.1
HD	1	17	8	30	68	38	0.79	4.56	13.82	170	30.5	12.3	31.4	52.4	3.7	8.5	3.4	0.7	0.8
HD	3	19	8	35	72	37	0.95	7.03	14.02	175	31	12.5	32.6	56	4.1	2.6	4.3	0.5	2.0
HD	3	27	8	29	62	33	0.88	6.68	13.07	153	29.7	11.7	25.1	66.4	3.3	2.1	2.3	0.8	-2.9
HD	3	30	8	29	67	38	0.76	6.27	12.6	154	30.4	12.2	30.3	55.7	5.9	4.2	2.7	1.2	0.9
HD	1	42	8	32	71	39	0.82	6.95	12.41	147	29.7	11.8	30	58.9	3.1	4.4	3.1	0.6	1.8
HD	3	92	8	25	60	35	0.71	3.98	12.47	149	28.6	12	30.3	52.2	5	8.4	3.2	0.9	2.0
HD	3	115	8	27	74	47	0.57	3.25	12.2	169	35.6	13.8	32.1	54.4	2	8.1	3.1	0.2	-1.4
Control	7	1	15	28	57	29	0.97	5.36	11.78	139	29.3	11.8	39.2	55.1	3.2	0.4	1.7	0.5	0.1
Control	8	3	15	33	60	27	1.22	4.87	11.92	148	30.8	12.4	37.1	56.6	3.2	0.4	2.3	0.4	-1.0
Control	7	10	15	33	64	31	1.06	6.06	10.82	145	33.1	13.4	34.1	55.7	8.8	0.1	0.9	0.4	0.2
Control	8	11	15	33	62	29	1.14	4.74	10.56	139	33.1	13.1	31.1	62.3	3.6	0.3	2.1	0.5	0.9
Control	7	14	15	35	70	35	1	6.06	11.82	141	30.1	11.9	34.7	59	3.6	0.3	1.8	0.6	1.9
Control	7	15	15	36	64	28	1.29	6.76	12.96	162	31.4	12.5	20	74.5	3.6	0.1	1.4	0.4	-1.6
Control	8	18	15	33	64	31	1.06	6.83	10.33	130	31.1	12.6	30.9	64.1	2.6	0.2	2	0.2	-0.2
Control	8	21	15	35	63	28	1.25	10.66	11.17	145	31.8	13	35.3	61.3	2	0.1	1.1	0.2	-1.0
Control	7	24	15	31	59	28	1.11	11.45	9.64	123	31.6	12.8	37.9	56.4	3.2	0.2	2	0.3	1.0
Control	8	26	15	35	62	27	1.3	7.67	13.32	165	30.6	12.4	30.3	65.9	2.4	0.2	1.1	0.2	-1.0
Control	7	47	15	28	53	25	1.12	4.91	10.03	153	37.3	15.3	10.6	79	6.7	0.1	2	1.7	1.1
Control	8	61	15	38	72	34	1.12	5.9	10.73	159	36.1	14.8	39.4	53.2	4.9	0.2	2	0.3	0.7
LD	5	5	15	30	64	34	0.88	6.98	12.06	163	33	13.5	25.6	66	1.9	2.2	3.7	0.6	-1.1
LD	5	6	15	24	57	33	0.73	.	.	.	.	.	.	.	.	.	.	.	-0.9
LD	5	13	15	32	67	35	0.91	9.05	9.69	115	29.1	11.9	37.1	53.2	2	2.2	5	0.5	-2.5
LD	5	20	15	35	64	29	1.21	7.66	10.89	138	31.1	12.7	34.6	55.1	5.5	1.6	2.8	0.4	4.0
LD	6	22	15	32	65	33	0.97	13.05	12.51	153	29.7	12.2	30.2	59.3	5.1	1.4	3.7	0.3	-0.2
LD	6	23	15	35	63	28	1.25	4.64	10.46	114	26.7	10.9	26.6	59.1	6.2	4.3	3	0.8	2.9
LD	5	38	15	34	79	45	0.76	6.98	13.09	157	28.8	12	28.5	53.4	4	10.6	3.2	0.3	1.3

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
LD	6	53	15	30	67	37	0.81	10.55	9.96	139	34.8	13.9	17.1	63.8	3.5	15.5	6.7	0.2	1.8
LD	6	56	15	32	66	34	0.94	8.05	11.22	147	32.1	13.1	38.3	50.5	3.2	2.7	4.8	0.4	0.6
LD	6	68	15	32	81	49	0.65	8.72	11.83	151	32.5	12.7	22	63.9	7.9	5.7	7.6	0.5	2.7
LD	5	104	15	30	66	36	0.83	6.91	12.78	157	30.2	12.3	24.2	61.7	4.3	8.1	1	0.6	6.7
MD	4	4	15	27	71	44	0.61	5.73	16.66	202	28.6	12.1	26.4	65.3	2.2	2.5	3.2	0.4	-0.3
MD	2	8	15	29	65	36	0.81	6.39	13.74	164	30.3	12	35.5	56.9	2.5	1.6	3.3	0.3	0.7
MD	4	16	15	32	75	43	0.74	6.44	13.03	159	30.3	12.2	53.1	41.5	2.7	1.2	1.2	0.3	0.8
MD	4	25	15	29	62	33	0.88	7.26	11.46	155	33.6	13.6	33.7	56.8	5.6	1.5	1.1	1.3	1.2
MD	4	28	15	31	69	38	0.82	7.17	12.58	149	28.7	11.8	24.6	66.1	3.4	1.9	3.5	0.5	0.8
MD	2	29	15	26	64	38	0.68	8.41	12.46	154	30.9	12.4	28.4	53.8	6.2	8.1	2.8	0.6	0.6
MD	4	39	15	28	72	44	0.64	5.4	12.88	188	35.9	14.6	20.4	62.7	2.2	11.3	3.1	0.4	3.1
MD	2	78	15	32	70	38	0.84	5.19	12.16	159	31.2	13	30.7	42.4	2.3	21.1	3	0.4	0.8
MD	4	81	15	26	64	38	0.68	9.09	11.39	146	31.6	12.8	29.4	64.2	2.6	3.6	5.6	0.2	2.4
MD	2	88	15	29	71	42	0.69	5.69	13.04	171	31.3	13.1	31	59.6	3	6.2	5.7	0.2	1.6
MD	2	98	15	25	63	38	0.66	5.43	10.83	140	32.4	12.9	27.3	65.2	3.9	1.7	1.3	0.7	-0.4
HD	1	2	15	32	69	37	0.86	8.85	11.19	151	32.9	13.5	28.4	59	7.3	4.4	9.5	0.9	0.1
HD	3	7	15	26	65	39	0.67	10.25	13.01	178	33.1	13.7	27.3	55.8	9.1	7.5	10.8	0.4	0.9
HD	1	9	15	28	67	39	0.72	7.09	13.64	176	31.2	12.9	38.4	50.1	2.7	4.4	3.9	0.6	-1.8
HD	1	12	15	26	70	44	0.59	4.69	11.88	154	32.5	13	39.4	48.7	5.4	2.2	3.6	0.7	-0.6
HD	1	17	15	28	68	40	0.7	6.79	13.49	166	30.3	12.3	30.7	57.1	2.5	4.9	4.5	0.3	1.6
HD	3	19	15	29	65	36	0.81	10.77	12.93	169	30.6	13.1	25.4	65.2	2.8	6.3	9.5	0.4	2.8
HD	3	27	15	28	60	32	0.88	8.11	13.56	165	29.5	12.2	27.7	63.8	4.5	2	1.9	0.1	13.0
HD	3	30	15	28	70	42	0.67	.	.	.	.	.	.	.	.	.	.	.	2.7
HD	1	42	15	29	70	41	0.71	8.98	12.32	147	29.5	11.9	38.7	50.4	3.9	6.4	5.4	0.6	1.2
HD	3	92	15	22	56	34	0.65	6.7	11.85	139	28.4	11.8	24.5	64.2	4.1	3.8	2.7	0.7	2.2
HD	3	115	15	23	73	50	0.46	8.58	11.53	163	35	14.2	39	53.6	3.1	1.5	2.6	0.2	6.4
Control	7	1	22	27	55	28	0.96	4.88	11.32	134	29.5	11.8	31.7	61.4	3.2	1.7	1.6	0.3	2.1
Control	8	3	22	33	59	26	1.27	7.02	11.53	140	30.9	12.2	34.2	57.6	6	0.4	1.7	0.2	1.6
Control	7	10	22	33	64	31	1.06	5.32	11.16	151	33.3	13.6	22.2	69.9	6.1	0.1	1.1	0.6	-0.1

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
Control	8	11	22	35	63	28	1.25	6.13	10.89	141	33.1	13	26.6	65.8	4.8	0.3	2.1	0.3	-2.2
Control	7	14	22	35	72	37	0.95	5.59	11.71	141	30.3	12	32	62.2	3.3	0.2	1.8	0.4	-2.1
Control	7	15	22	36	63	27	1.33	7.61	12.52	154	31.6	12.3	20.1	74.3	4.1	0.2	1.1	0.3	-2.1
Control	8	18	22	32	67	35	0.91	7.14	10.03	125	31.9	12.5	35.6	53.6	8.1	0.2	2.3	0.1	3.9
Control	8	21	22	35	62	27	1.3	10.1	10.78	134	31.5	12.5	29.4	65.5	3.1	0.3	1.5	0.2	4.2
Control	7	24	22	33	61	28	1.18	9.5	10.86	136	31.9	12.5	31.1	62.3	3.5	0.2	2.6	0.2	3.0
Control	8	26	22	33	59	26	1.27	7.26	11.77	139	29.2	11.8	28.1	65.6	4.5	0.2	1.4	0.3	5.3
Control	7	47	22	31	56	25	1.24	4.1	10.89	161	37	14.8	15.1	77	5.1	0.2	1.4	1.1	3.7
Control	8	61	22	39	70	31	1.26	5.79	10.13	146	35.7	14.4	34.2	58.7	4.4	0.3	2.2	0.2	1.8
LD	5	5	22	28	60	32	0.88	7.41	10.62	139	32.8	13.1	24.3	65.2	5.3	1.7	3.2	0.3	-1.5
LD	5	6	22	25	56	31	0.81	6.84	10.02	125	32.1	12.5	19.1	71	3.2	2.7	3.4	0.6	-0.7
LD	5	13	22	32	67	35	0.91	8.58	10.55	125	29	11.9	31.9	60.7	4.5	2.5	5	0.4	-5.9
LD	5	20	22	34	64	30	1.13	7.6	10.77	134	31.2	12.4	28.3	59.9	5.2	1.5	4.6	0.5	5.5
LD	6	22	22	33	68	35	0.94	12.49	13.48	162	30	12	27.8	66.6	2.7	2.7	6.1	0.2	1.2
LD	6	23	22	35	62	27	1.3	6.6	9.64	108	26.9	11.2	19.7	64	6.1	5.6	3.9	0.7	-2.4
LD	5	38	22	33	76	43	0.77	8.37	12.77	148	28.9	11.6	25.4	58.7	2.5	9.2	3.9	0.2	2.1
LD	6	53	22	31	68	37	0.84	9.14	10.52	143	34.8	13.6	20.3	66.2	3.1	10.1	6.8	0.4	-1.6
LD	6	56	22	31	56	34	0.91	7.99	11.58	146	32.4	12.6	33.3	55	4	2.8	4.5	0.4	0.1
LD	6	68	22	31	75	44	0.7	10.64	10.14	124	32	12.3	21.6	69	3.9	5	6.5	0.5	-0.2
LD	5	104	22	30	66	36	0.83	7.1	12.89	160	30.2	12.4	20.6	68.6	2.6	7.1	0.8	0.3	1.4
MD	4	4	22	25	69	44	0.57	5.6	13.44	155	28.1	11.6	28.3	60.9	3.8	2.5	3.8	0.8	-0.2
MD	2	8	22	27	64	37	0.73	9.12	12.63	150	30	11.8	34.3	61.4	2.5	1.5	5.1	0.3	0.2
MD	4	16	22	27	72	45	0.6	13.42	11.88	141	30.1	11.8	49.5	44.8	2.4	1.5	1.4	0.4	4.2
MD	4	25	22	28	60	32	0.88	9.26	11.15	144	33.2	12.9	29.1	63	3.4	1.5	2.2	0.8	0.2
MD	4	28	22	31	67	36	0.86	9.14	11.38	133	28.7	11.7	22.7	68.5	2.7	1.4	4.3	0.4	-0.5
MD	2	29	22	22	57	35	0.63	8.39	10.93	129	30.9	11.8	26.2	61.8	3.8	5	2.3	0.8	2.7
MD	4	39	22	27	68	41	0.66	6.06	11.29	162	35.4	14.4	26.6	60.3	4.3	8.3	5.7	0.5	0.9
MD	2	78	22	34	71	37	0.92	5.12	12.65	162	31.2	12.8	29.5	57.2	3.2	6.7	3.3	0.2	-3.3
MD	4	81	22	26	63	37	0.7	9.19	10.89	133	31.2	12.2	25.1	65	1	4.8	3.9	0.2	-3.8

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
MD	2	88	22	26	63	37	0.7	7.72	9.76	119	30.5	12.2	35.7	51.9	3.7	8.6	8.2	0.2	-0.9
MD	2	98	22	25	63	38	0.66	6.43	10.66	141	32.5	13.2	31.1	58.7	3.4	3.8	2.5	0.5	-1.4
HD	1	2	22	30	69	39	0.77	8.02	11.42	150	32.9	13.1	26	67.2	5	1.3	10.3	0.4	8.2
HD	3	7	22	24	63	39	0.62	12.06	14.5	191	33.1	13.2	20.2	67.6	8.6	2.9	13.9	0.6	1.2
HD	1	9	22	26	63	37	0.7	6.96	13.24	168	30.9	12.7	51.6	37.8	4.4	2.2	3.4	0.5	2.0
HD	1	12	22	24	69	45	0.53	8.83	11.83	154	32.2	13	38.5	53.9	3.5	0.9	2.4	0.9	-3.1
HD	1	17	22	29	70	41	0.71	6.62	13.64	168	30.5	12.3	29.3	57.6	2.6	5.8	4.2	0.5	-3.4
HD	3	19	22	30	68	38	0.79	14.27	13.51	168	30.9	12.5	25.9	67	5.4	1.5	11.1	0.3	-0.3
HD	3	27	22	25	58	33	0.76	9.46	12.95	154	29	11.9	24.4	68	3.3	1.1	1.9	1.3	3.5
HD	3	30	22	27	70	43	0.63	9.34	11.11	137	30.5	12.3	27.2	62.9	3.1	2.5	3.7	0.6	-1.2
HD	1	42	22	29	70	41	0.71	7.03	11.94	141	29.4	11.8	29.5	56.3	4.1	9.4	8.8	0.5	1.8
HD	3	92	22	20	53	33	0.61	4.8	10.9	125	28.3	11.5	25.1	63.4	5.4	3.3	2.3	0.5	-5.7
HD	3	115	22	20	63	43	0.47	5.16	11.65	157	34.8	13.5	29.4	64.6	1.8	1.7	2.1	0.4	-0.7
Control	7	1	29	28	58	30	0.93	4.87	10.82	124	29.2	11.5	44	48.9	3.7	0.6	2	0.7	-3.8
Control	8	3	29	33	59	26	1.27	6.37	11.63	143	30.8	12.3	34.9	59.4	2.9	0.3	2.2	0.3	2.4
Control	7	10	29	33	62	29	1.14	5.2	10.04	135	33	13.4	29.1	60.9	8.5	0.2	0.9	0.5	1.7
Control	8	11	29	36	66	30	1.2	5.09	11.2	145	33.1	12.9	24.8	69.9	2.4	0.2	2.6	0.1	6.7
Control	7	14	29	36	71	35	1.03	5.61	11.72	140	30.5	11.9	29.6	65.4	2.7	0.2	1.9	0.2	7.2
Control	7	15	29	36	62	26	1.38	7.03	12.81	157	31.4	12.3	18.2	74.3	5.6	0.2	1.5	0.3	9.6
Control	8	18	29	31	69	38	0.82	6.25	10.31	129	31.9	12.5	30.6	61.8	4.5	0.1	2.7	0.2	6.3
Control	8	21	29	36	63	27	1.33	9.06	9.42	116	30.9	12.3	31.3	64.8	2.3	0.2	1.3	0.1	3.3
Control	7	24	29	34	63	29	1.17	8.36	10.67	133	31.5	12.5	27.5	66	4	0.1	2.2	0.2	5.3
Control	8	26	29	34	59	26	1.36	7.23	12.04	142	29.1	11.8	29.6	64.2	4.6	0.2	1.3	0.3	6.8
Control	7	47	29	31	56	25	1.24	3.5	9.52	137	35.9	14.4	11	77.6	7.2	0.3	3.4	0.5	3.5
Control	8	61	29	40	72	32	1.25	5.66	11.3	164	35.7	14.5	33	55.6	9	0.3	1.9	0.2	2.7
LD	5	5	29	29	60	31	0.94	8.75	11.09	145	32.7	13.1	21	71.2	3.1	1.6	2.8	0.3	-4.7
LD	5	6	29	23	55	32	0.72	6.75	8.64	107	31.6	12.4	17.4	68.1	4.6	5.5	3.9	0.5	4.7
LD	5	13	29	31	68	37	0.84	6.98	11.86	140	29.7	11.8	30.7	59.9	3.1	2.6	3.2	0.4	0.9
LD	5	20	29	35	65	30	1.17	6.44	11.1	138	31.9	12.4	30	60	3.3	2.1	4.2	0.4	2.5

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
LD	6	22	29	32	66	34	0.94	10.33	11.93	139	29.7	11.7	24.5	63.5	4.1	2.8	4.8	0.2	4.4
LD	6	23	29	34	63	29	1.17	6.1	11.23	126	28	11.2	17.2	67.3	2.8	8.5	3.6	0.6	4.1
LD	5	38	29	34	77	43	0.79	6.99	13.26	153	28.8	11.5	26.2	59.6	5.1	5.7	3.1	0.2	3.4
LD	6	53	29	31	69	38	0.82	7.97	10.95	151	35	13	18.7	70.7	3.5	6.9	5.7	0.3	6.1
LD	6	56	29	30	61	31	0.97	7.86	12.36	162	32.6	13.1	35.1	52.3	4.3	4.2	3.6	0.5	-1.6
LD	6	68	29	32	76	44	0.73	9.63	10.82	136	32.7	12.6	21.9	69.2	4.1	4.5	5.4	0.4	1.7
LD	5	104	29	31	68	37	0.84	5.56	14.06	172	30.1	12.2	21	70.9	2.4	3.9	1.1	0.6	4.0
MD	4	4	29	24	69	45	0.53	4.66	15.69	180	28.2	11.5	18.8	71.8	6.5	1.9	5.2	1.9	-6.1
MD	2	8	29	23	59	36	0.64	6.89	14.32	170	30.1	11.9	38.5	52	3.6	1.8	3.5	0.6	1.4
MD	4	16	29	27	69	42	0.64	7.89	13.47	164	30.6	12.2	33.1	59	1.8	4.1	1.8	0.4	4.6
MD	4	25	29	25	54	31	0.74	7.7	11.56	151	33.2	13.1	21.6	69.7	4.9	1.3	1.6	0.9	7.7
MD	4	28	29	31	68	37	0.84	9.51	13.6	166	30.2	12.2	26.5	63.6	3.7	2.1	3.8	0.4	6.0
MD	2	29	29	23	58	35	0.66	8.91	11.4	134	31.6	11.8	26.7	60.9	3.6	5.9	2.3	0.4	3.9
MD	4	39	29	26	70	44	0.59	5.68	11.69	166	35.3	14.2	17.1	68.7	6.5	7.4	5.1	0.4	10.0
MD	2	78	29	32	72	40	0.8	4.61	13.51	172	31.2	12.7	25.9	54.7	9.8	6	3.3	0.4	7.3
MD	4	81	29	22	57	35	0.63	5.11	9.9	122	30.3	12.3	36.8	56.1	2.6	0.8	2.6	1	1.0
MD	2	88	29	24	59	35	0.69	6.54	9.28	112	30.5	12.1	41.3	47.5	4.9	5.9	5.9	0.3	1.5
MD	2	98	29	24	64	40	0.6	5.24	12.54	168	33.1	13.4	18.5	63.3	11.3	1.4	5	0.5	3.6
HD	1	2	29	28	67	39	0.72	.	.	.	.	.	.	.	.	.	.	.	4.7
HD	3	7	29	18	46	28	0.64	.	.	.	.	.	.	.	.	.	.	.	2.7
HD	1	9	29	24	60	36	0.67	.	.	.	.	.	.	.	.	.	.	.	22.0
HD	1	12	29	24	71	47	0.51	.	.	.	.	.	.	.	.	.	.	.	4.6
HD	1	17	29	26	64	38	0.68	.	.	.	.	.	.	.	.	.	.	.	2.9
HD	3	19	29	24	58	34	0.71	.	.	.	.	.	.	.	.	.	.	.	0.9
HD	3	27	29	24	57	33	0.73	.	.	.	.	.	.	.	.	.	.	.	8.8
HD	3	30	29	26	69	43	0.6	.	.	.	.	.	.	.	.	.	.	.	7.8
HD	1	42	29	25	59	34	0.74	.	.	.	.	.	.	.	.	.	.	.	-1.2
HD	3	92	29	18	48	30	0.6	.	.	.	.	.	.	.	.	.	.	.	4.8
HD	3	115	29	19	60	41	0.46	.	.	.	.	.	.	.	.	.	.	.	7.5

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Mcv	Mch	Neut	Lymph	Mono	Eosi	Baso	Luc	Pep
Control	7	1	36	28	58	30	0.93	5.29	11.98	138	29.6	11.5	38.2	56.4	3.1	0.6	1.4	0.3	1.0
Control	8	3	36	35	64	29	1.21	5.24	11.68	150	32.1	12.8	36.9	58.1	1.7	0.4	2.7	0.2	0.8
Control	7	10	36	35	69	34	1.03	5.28	11.73	166	34.9	14.1	30.5	65	3	0.2	0.9	0.4	2.0
Control	8	11	36	37	65	28	1.32	4.19	10.98	147	33.6	13.4	29.6	61.6	2.8	3.5	2.2	0.2	1.7
Control	7	14	36	38	75	37	1.03	5.14	12.63	153	30.7	12.1	33.1	60.8	2.8	0.4	2.5	0.5	-0.1
Control	7	15	36	37	64	27	1.37	6.96	13.34	166	31.9	12.5	17.8	77.4	2.9	0.3	1.4	0.1	1.6
Control	8	18	36	33	66	33	1	5.83	9.81	126	31.7	12.9	35.3	59.3	2.8	0.2	2.3	0.1	2.7
Control	8	21	36	37	66	29	1.28	8.38	9.82	129	32.1	13	39.9	53.2	4.6	1.1	1	0.2	0.1
Control	7	24	36	34	64	30	1.13	8.25	10.03	129	31.9	12.8	29.6	64.9	2.6	0.3	2.5	0.1	5.8
Control	8	26	36	36	63	27	1.33	6.82	12.32	146	29.5	11.8	34.4	62.5	1.7	0.3	0.8	0.3	10.5
Control	7	47	36	32	59	27	1.19	3.24	9.3	135	35.7	14.5	10.2	79.9	5.5	0.6	3.1	0.7	1.7
Control	8	61	36	41	73	32	1.28	5.22	10.09	143	35.3	14.2	37.9	55.5	4	0.3	2.2	0.1	2.2
LD	5	5	36	24	51	27	0.89	5.74	9.89	128	31.9	12.9	13.5	78.9	3.2	1.1	2.8	0.5	2.6
LD	5	6	36	21	50	29	0.72	5.1	9.23	117	31	12.7	7.3	80.3	3.4	5.1	3.6	0.2	3.4
LD	5	13	36	26	58	32	0.81	5.96	10.79	129	29.2	12	15.9	75.2	7.3	1.2	9.1	0.3	0.9
LD	5	20	36	37	72	35	1.06	7.05	11.69	151	32.8	12.9	28.9	66.9	3.2	0.8	5.9	0.1	0.6
LD	6	22	36	34	68	34	1	10.11	10.91	127	29	11.7	18.6	72.9	3.6	0.9	3.8	0.2	4.6
LD	6	23	36	34	65	31	1.1	4.76	10.64	122	28.6	11.5	12	77.7	4.3	5.6	5.6	0.4	6.7
LD	5	38	36	34	78	44	0.77	6.54	13.13	152	28.5	11.6	21.8	62.8	9.5	1.8	3.9	0.2	-0.4
LD	6	53	36	32	70	38	0.84	6.39	11.53	162	35.6	14	14.7	76.9	3.2	5	6.5	0.2	4.8
LD	6	56	36	20	49	29	0.69	4.98	13.06	167	32.2	12.8	16	77.7	4.6	1.5	5.2	0.3	7.1
LD	6	68	36	33	79	46	0.72	9.74	10.25	132	33.3	12.8	24.1	67.4	7	1.2	7.2	0.3	6.3
LD	5	104	36	32	74	42	0.76	7.32	13.53	162	29.6	12	24	70.8	2.3	1.2	1.1	0.7	2.1

2.2 Data- Faecal egg counts and Liveweight.

Treat= Treatment group, LD= Low dose, MD= Medium dose, HD= High dose, Pen= Allocation of the deer on the pen, Anim= Identification number of the deer, Day= time before and after beginning of trickle infection, FEC= Faecal egg count (eggs per gram), LW= Live weight (Kg).

Treat	Pen	Anim	Day	Fec	LW
Control	7	1	-14	0	29
Control	8	3	-14	0	36
Control	7	10	-14	0	48.5
Control	8	11	-14	0	40
Control	7	14	-14	0	43.5
Control	7	15	-14	0	39
Control	8	18	-14	0	46.5
Control	8	21	-14	0	37.5
Control	7	24	-14	0	50
Control	8	26	-14	0	42.5
Control	7	47	-14	0	33
Control	8	61	-14	0	35.5
LD	5	5	-14	0	46
LD	5	6	-14	0	34.5
LD	5	13	-14	0	39
LD	5	20	-14	0	43
LD	6	22	-14	0	48.5
LD	6	23	-14	0	50

Treat	Pen	Anim	Day	Fec	LW
LD	5	38	-14	0	42.5
LD	6	53	-14	0	37.5
LD	6	56	-14	0	32
LD	6	68	-14	0	36
LD	5	104	-14	0	40
MD	4	4	-14	0	48
MD	2	8	-14	0	35.5
MD	4	16	-14	0	43.5
MD	4	25	-14	0	33.5
MD	4	28	-14	0	42.5
MD	2	29	-14	0	49
MD	4	39	-14	0	39.5
MD	2	78	-14	0	36.5
MD	4	81	-14	0	30
MD	2	88	-14	0	38.5
MD	2	98	-14	0	40.5
HD	1	2	-14	0	43
HD	3	7	-14	0	35.5
HD	1	9	-14	0	34.5
HD	1	12	-14	0	40
HD	1	17	-14	0	48.5
HD	3	19	-14	0	49
HD	3	27	-14	0	44.5
HD	3	30	-14	0	39

Treat	Pen	Anim	Day	Fec	LW
HD	1	42	-14	0	37
HD	3	92	-14	0	41.5
HD	3	115	-14	0	31.5
Control	7	1	1	0	29
Control	8	3	1	0	38
Control	7	10	1	0	49.5
Control	8	11	1	0	40
Control	7	14	1	0	45
Control	7	15	1	0	39
Control	8	18	1	0	47.5
Control	8	21	1	0	39.5
Control	7	24	1	0	52
Control	8	26	1	0	48
Control	7	47	1	0	31.5
Control	8	61	1	0	37.5
LD	5	5	1	0	45
LD	5	6	1	0	36
LD	5	13	1	0	40.5
LD	5	20	1	0	45.5
LD	6	22	1	0	50
LD	6	23	1	0	52
LD	5	38	1	0	42.5
LD	6	53	1	0	38
LD	6	56	1	0	33.5

Treat	Pen	Anim	Day	Fec	LW
LD	6	68	1	0	38.5
LD	5	104	1	0	42
MD	4	4	1	0	50
MD	2	8	1	0	37.5
MD	4	16	1	0	45.5
MD	4	25	1	0	37
MD	4	28	1	0	46.5
MD	2	29	1	0	51
MD	4	39	1	0	40.5
MD	2	78	1	0	39.5
MD	4	81	1	0	31.5
MD	2	88	1	0	39.5
MD	2	98	1	0	41.5
HD	1	2	1	0	44
HD	3	7	1	0	36
HD	1	9	1	0	35.5
HD	1	12	1	0	41.5
HD	1	17	1	0	49.5
HD	3	19	1	0	50
HD	3	27	1	0	45
HD	3	30	1	0	42
HD	1	42	1	0	38.5
HD	3	92	1	0	40.5
HD	3	115	1	0	35

Treat	Pen	Anim	Day	Fec	LW
Control	7	1	8	0	28.5
Control	8	3	8	0	38
Control	7	10	8	0	51
Control	8	11	8	0	41
Control	7	14	8	0	47
Control	7	15	8	0	42
Control	8	18	8	0	49.5
Control	8	21	8	0	40
Control	7	24	8	0	53
Control	8	26	8	0	49
Control	7	47	8	0	31.5
Control	8	61	8	0	38
LD	5	5	8	0	45.5
LD	5	6	8	0	36
LD	5	13	8	0	39
LD	5	20	8	0	47
LD	6	22	8	0	51
LD	6	23	8	0	51
LD	5	38	8	0	45
LD	6	53	8	0	38.5
LD	6	56	8	0	34
LD	6	68	8	0	38
LD	5	104	8	0	42
MD	4	4	8	0	47.5

Treat	Pen	Anim	Day	Fec	LW
MD	2	8	8	0	36.5
MD	4	16	8	0	46
MD	4	25	8	0	36.5
MD	4	28	8	0	46
MD	2	29	8	0	50.5
MD	4	39	8	0	40.5
MD	2	78	8	0	38.5
MD	4	81	8	0	29.5
MD	2	88	8	0	38
MD	2	98	8	0	40
HD	1	2	8	0	44
HD	3	7	8	0	34
HD	1	9	8	0	34.5
HD	1	12	8	0	38.5
HD	1	17	8	0	48
HD	3	19	8	0	48.5
HD	3	27	8	0	43.5
HD	3	30	8	0	40.5
HD	1	42	8	0	37.5
HD	3	92	8	0	39.5
HD	3	115	8	0	34
Control	7	1	15	0	29.5
Control	8	3	15	0	39.5
Control	7	10	15	0	52.5

Treat	Pen	Anim	Day	Fec	LW
Control	8	11	15	0	42.5
Control	7	14	15	0	48.5
Control	7	15	15	0	43
Control	8	18	15	0	50.5
Control	8	21	15	0	41.5
Control	7	24	15	0	56.5
Control	8	26	15	0	51
Control	7	47	15	0	31.5
Control	8	61	15	0	39.5
LD	5	5	15	0	47
LD	5	6	15	0	35
LD	5	13	15	0	41
LD	5	20	15	0	49
LD	6	22	15	0	52
LD	6	23	15	0	53
LD	5	38	15	0	47.5
LD	6	53	15	0	40
LD	6	56	15	0	35
LD	6	68	15	0	40
LD	5	104	15	0	42
MD	4	4	15	0	48
MD	2	8	15	0	36.5
MD	4	16	15	0	46
MD	4	25	15	0	37.5

Treat	Pen	Anim	Day	Fec	LW
MD	4	28	15	0	47.5
MD	2	29	15	0	51.5
MD	4	39	15	0	41.5
MD	2	78	15	0	39.5
MD	4	81	15	0	29.5
MD	2	88	15	0	38
MD	2	98	15	0	40
HD	1	2	15	0	44.5
HD	3	7	15	0	35
HD	1	9	15	0	34.5
HD	1	12	15	0	39
HD	1	17	15	50	49
HD	3	19	15	0	49.5
HD	3	27	15	0	43.5
HD	3	30	15	0	42
HD	1	42	15	0	38.5
HD	3	92	15	0	40
HD	3	115	15	0	34
Control	7	1	22	0	29.5
Control	8	3	22	0	40.5
Control	7	10	22	0	53
Control	8	11	22	0	43
Control	7	14	22	0	49.5
Control	7	15	22	0	43.5

Treat	Pen	Anim	Day	Fec	LW
Control	8	18	22	0	50.5
Control	8	21	22	0	41.5
Control	7	24	22	0	56
Control	8	26	22	0	52.5
Control	7	47	22	0	31
Control	8	61	22	0	40
LD	5	5	22	0	45.5
LD	5	6	22	200	36
LD	5	13	22	50	41
LD	5	20	22	0	49
LD	6	22	22	50	53
LD	6	23	22	100	54.5
LD	5	38	22	50	48.5
LD	6	53	22	50	41.5
LD	6	56	22	250	34.5
LD	6	68	22		41.5
LD	5	104	22	50	44
MD	4	4	22	0	48.5
MD	2	8	22	50	35.5
MD	4	16	22	200	47
MD	4	25	22	100	39
MD	4	28	22	50	49.5
MD	2	29	22	300	50.5
MD	4	39	22	50	42.5

Treat	Pen	Anim	Day	Fec	LW
MD	2	78	22	50	40.5
MD	4	81	22	50	29.5
MD	2	88	22	200	37.5
MD	2	98	22	150	40
HD	1	2	22		44.5
HD	3	7	22	1050	33.5
HD	1	9	22	200	33.5
HD	1	12	22	250	38
HD	1	17	22	50	50.5
HD	3	19	22	250	48
HD	3	27	22	500	42.5
HD	3	30	22		43
HD	1	42	22	250	38.5
HD	3	92	22	750	39.5
HD	3	115	22	150	33.5
Control	7	1	29	0	29.5
Control	8	3	29	0	40.5
Control	7	10	29	0	53.5
Control	8	11	29	0	43.5
Control	7	14	29	0	49
Control	7	15	29	0	44.5
Control	8	18	29	0	49.5
Control	8	21	29	0	42
Control	7	24	29	0	57

Treat	Pen	Anim	Day	Fec	LW
Control	8	26	29	0	52.5
Control	7	47	29	0	32
Control	8	61	29	0	41
LD	5	5	29	150	45.5
LD	5	6	29	300	35.5
LD	5	13	29	450	40
LD	5	20	29	100	49.5
LD	6	22	29	150	53
LD	6	23	29	150	55
LD	5	38	29	0	49
LD	6	53	29	50	41.5
LD	6	56	29	200	35
LD	6	68	29	50	41
LD	5	104	29	200	44.5
MD	4	4	29	150	45.5
MD	2	8	29	500	34
MD	4	16	29	150	46.5
MD	4	25	29	200	38.5
MD	4	28	29	150	49.5
MD	2	29	29	300	50
MD	4	39	29	100	42
MD	2	78	29	150	39.5
MD	4	81	29		28.5
MD	2	88	29	300	37.5

Treat	Pen	Anim	Day	Fec	LW
MD	2	98	29	800	37
HD	1	2	29	800	.
HD	3	7	29	2100	.
HD	1	9	29	500	.
HD	1	12	29	1350	.
HD	1	17	29	50	.
HD	3	19	29	1150	.
HD	3	27	29	1250	.
HD	3	30	29	300	.
HD	1	42	29	200	.
HD	3	92	29	1000	.
HD	3	115	29	350	.
Control	7	1	36	0	29
Control	8	3	36		40.5
Control	7	10	36	0	54
Control	8	11	36	0	43.5
Control	7	14	36	0	50.5
Control	7	15	36	0	44.5
Control	8	18	36	0	51
Control	8	21	36	0	43
Control	7	24	36	0	56
Control	8	26	36	0	52.5
Control	7	47	36	0	32.5
Control	8	61	36	0	41

Treat	Pen	Anim	Day	Fec	LW
LD	5	5	36	0	44
LD	5	6	36	500	35
LD	5	13	36	1600	38
LD	5	20	36	0	50
LD	6	22	36	400	53.5
LD	6	23	36	650	54
LD	5	38	36	150	49.5
LD	6	53	36	250	42
LD	6	56	36	350	32.5
LD	6	68	36	450	41.5
LD	5	104	36	300	44

2.3 Data-Feed intake.

Treat= Treatment group, LD= Low dose, MD= Medium dose, HD= High dose, Pen= Allocation of the deer on the pen, Anim= Identification number of the deer, Day n= intake (kg/head/day) of the group of deer in the pen, before (Day 0) and after (Day 1-38) beginning of trickle infection.

Treat	Pen	Anim	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Control	7	1,10,14,15,24,47	1.297	1.311	1.273	1.296	1.27	1.265	1.259	0.711	1.306	1.254	1.239	1.237	1.257	1.287
Control	8	3,11,18,21,26,61	1.510	1.52	1.468	1.504	1.481	1.413	1.395	0.787	1.443	1.483	1.525	1.51	1.499	1.535
LD	5	5,6,13,20,38,104	1.470	1.457	1.423	1.454	1.348	1.361	1.358	0.749	1.387	1.276	1.331	1.225	1.272	1.314
LD	6	22,23,53,56,68	1.472	1.429	1.396	1.45	1.381	1.307	1.335	0.761	1.394	1.317	1.366	1.284	1.337	1.395
MD	2	8,29,78,88,98	1.378	1.23	1.181	1.25	1.186	1.141	1.17	0.67	1.138	1.025	1.133	0.924	1.042	1.028
MD	4	4,16,25,28,39,81	1.453	1.392	1.315	1.362	1.288	1.253	1.259	0.711	1.234	1.15	1.255	1.112	1.232	1.217
HD	1	2,9,12,17,42	1.443	1.342	1.213	1.217	0.924	1.007	1.026	0.67	1.035	1.038	1.055	0.952	1.062	0.981
HD	3	7,19,27,30,92,115	1.411	1.247	1.118	1.135	1.109	1.089	1.124	0.634	1.045	1.023	1.014	0.912	0.978	0.951

Treat	Pen	Anim	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26
Control	7	1,10,14,15,24,47	1.285	1.16	1.278	1.27	1.235	1.218	1.265	1.287	1.216	1.282	1.254	1.239	1.21
Control	8	3,11,18,21,26,61	1.503	1.395	1.455	1.48	1.461	1.479	1.431	1.487	1.479	1.485	1.486	1.468	1.396
LD	5	5,6,13,20,38,104	1.28	1.252	1.269	1.283	1.3	1.336	1.353	1.349	1.307	1.324	1.317	1.297	1.203
LD	6	22,23,53,56,68	1.364	1.249	1.349	1.354	1.305	1.362	1.326	1.35	1.32	1.321	1.352	1.251	1.193
MD	2	8,29,78,88,98	0.954	0.823	0.941	0.931	0.933	1.015	0.94	0.981	0.902	0.972	0.953	0.793	0.799
MD	4	4,16,25,28,39,81	1.176	1.072	1.222	1.226	1.202	1.254	1.217	1.274	1.233	1.224	1.24	1.111	1.051
HD	1	2,9,12,17,42	0.891	0.812	1.013	0.941	0.957	1.011	0.995	0.898	0.933	0.938	0.9	0.737	0.752
HD	3	7,19,27,30,92,115	0.928	0.804	0.957	0.947	0.857	0.939	0.86	0.836	0.76	0.667	0.628	0.588	0.516

Treat	Pen	Anim	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38
Control	7	1,10,14,15,24,47	1.238	1.266	1.133	1.203	1.244	1.247	1.208	1.26	1.265	1.206	1.256	1.155
Control	8	3,11,18,21,26,61	1.402	1.419	1.365	1.402	1.442	1.437	1.413	1.429	1.456	1.427	1.456	1.412

LD	5	5,6,13,20,38,104	1.256	1.266	1.105	1.183	1.182	1.19	1.159	1.183	1.19	1.175	1.171	0.1
LD	6	22,23,53,56,68	1.263	1.277	1.216	1.103	1.284	1.19	1.093	1.195	1.157	1.188	1.189	0.12
MD	2	8,29,78,88,98	0.697	0.699	0.628	0.487	.	.	.	.	.	.	.	.
MD	4	4,16,25,28,39,81	1.035	0.974	0.891	0.927	.	.	.	.	.	.	.	.
HD	1	2,9,12,17,42	0.883	.	.	.	.	.	.	.	.	.	.	.
HD	3	7,19,27,30,92,115	0.504	.	.	.	.	.	.	.	.	.	.	.

2.4 Data- Nematode counts by genus and species.

Treat= Treatment group, LD= Low dose, MD= Medium dose, HD= High dose, Anim= Identification number of the deer, Oster= *Ostertagia*-type nematode, Spi= *Spiculopteragia spiculoptera,* Asy= *Spiculopteragia asymmetrica,* Lep= *Ostertagia leptospicularis*, Kol= Ostertagia kolchida, Circ= *Teladorsagia circumcincta*, Haem= *Haemonchus contortus*, Trich Abo= *Trichostrongylus* spp. abomasum, Larvae Abo= Larvae from the abomasum, Oeso= *Oesophagostomum* spp., Oven= *Oesophagostomum venulosum*, Osik= *Oesophagostomum sikae*, Larvae LI= Larvae from the large intestine, Trich SI= *Trichostrongylus* spp. from the small intestine, Coop= *Cooperia* spp.

Anim	Treat	Oster	Spi	Asy	Lep	Kol	Circ	Haem	Trich Abo	Larvae Abo	Oeso	Oven	Osik	Larvae LI	Trich SI	Coop
5	LD	1620	1114	354	101	51	0	40	0	140	760	760	0	740	.	.
6	LD	3320	1897	870	395	158	0	40	0	80	560	538	22	180	40	0
13	LD	2620	1310	721	524	66	0	60	0	120	460	460	0	220	40	0
20	LD	2780	1362	908	454	57	0	40	0	60	500	500	0	560	.	.
22	LD	1700	944	315	378	63	0	0	0	80	280	280	0	360	.	.
23	LD	2540	1575	660	254	0	51	120	20	160	700	700	0	820	.	.
38	LD	1820	993	717	55	55	0	20	0	160	600	600	0	180	.	.
53	LD	2640	1373	739	422	106	0	60	0	20	950	939	11	780	0	0
56	LD	2140	1213	713	143	0	71	20	0	100	680	680	0	900	20	0
68	LD	1480	770	592	118	0	0	40	0	20	380	380	0	240	0	
104	LD	2600	1040	715	845	0	0	40	0	120	920	878	42	1040	20	20
4	MD	5840	4555	818	467	0	0	60	0	860	740	687	53	1260	0	20
8	MD	7160	4010	1432	1575	0	143	160	20	360	1800	1755	45	2300	0	0
16	MD	6240	3744	1622	749	125	0	80	0	480	1420	1378	42	2300	40	0
25	MD	5820	3492	1862	466	0	0	120	0	600	800	800	0	1740	200	0
28	MD	5500	2750	1540	770	440	0	160	0	560	60	60	0	900	180	0

29	MD	7520	3760	2106	1053	602	0	200	0	1620	1240	1215	25	1780	60	0
39	MD	4180	2049	1311	656	82	82	120	0	180	1160	1119	41	1040	0	20
78	MD	5940	3584	1536	614	205	0	140	0	360	1860	1860	0	1400	0	40
81	MD	5720	2996	1798	708	218	0	100	0	1080	1580	1487	93	800	20	0
88	MD	5780	2555	1764	1217	243	0	0	0	640	860	688	172	940	20	0
98	MD	7420	4452	2078	742	148	0	180	0	680	1240	1240	0	1580	0	20
2	HD	11200	5051	3514	2196	439	0	140	0	2120	2600	2600	0	2760	20	0
7	HD	9280	4454	3155	1485	186	0	300	20	2840	1540	1540	0	4760	100	40
9	HD	9800	6045	2015	1465	183	92	160	0	2620	1420			2380	100	20
12	HD	9480	7271	1197	644	368	0	220	0	2860	1920	1804	116	2220	140	0
17	HD	8860	4430	2304	1949	177	0	60	0	1380	1660	1660	0	1140	0	20
19	HD	10700	5992	3424	1284	0	0	200	0	1280	1500	1500	0	3400	40	0
27	HD	10800	7128	2592	864	0	216	220	0	2160	3040	3040	0	3620	0	20
30	HD	11880	7603	2851	1188	238	0	200	0	2100	1440	1440	0	3820	40	0
42	HD	6420	3210	1284	1798	128	0	120	0	2020	2800	2800	0	2460	160	0
92	HD	10040	8032	0	2008	0	0	360	0	1560	1040	1019	21	4240	40	0
115	HD	9300	4464	1488	3348	0	0	120	0	1000	1680	1680	0	1440	40	0

Appendix 3 Supplementary information for Chapter 3. Analysis of the pathogenicity and diagnosis of GI nematode infections in young farmed deer – Supplementary follow-up study

1.1 Data- Blood parameters

Treat= Treatment Group, LrD= Lower dose, Cntr= Control, Pen= allocation of the deer on the pen, Anim= Identification number of the deer, Day= time before and after beginning of trickle infection, Alb= Albumin (g/l), TP= Total protein, Glob= Globulin (g/l), Agr= Albumin to globulin ratio, Wbc= White blood cell count (x 10^9 cells/l), Rbc= Red blood cell count (x 10^9 cells/l), Hgb= Haemoglobin (g/l), Pcv= Packed Cell Volume, Neut= Neutrophils (x10^9 cells/l), Lymph= Lymphocytes (x10^9 cells/l),
Mono= Monocytes (x10^9 cells/l), Eosi= Eosinophils (x10^9 cell/l), Baso= Basophils (x10^9 cell/l)

, Pep= Pepsinogen (iU).

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Pcv	Neut	Lymph	Mono	Eosi	Baso	Pep
Cntr	4	3	-7	36	61	25	1.44	5.53	11.08	148	0.38	1.94	3.25	0.18	0.03	0.13	-0.3
Cntr	3	11	-7	38	63	25	1.52	4.6	10.71	146	0.38	1.27	3.04	0.13	0.02	0.13	-1
Cntr	3	18	-7	39	69	30	1.3	6.86	10.99	145	0.37	1.92	4.42	0.25	0.01	0.24	-2.46
Cntr	4	21	-7	40	63	23	1.74	11.59	11.08	148	0.39	3.69	6.95	0.68	0.05	0.19	4.28
Cntr	3	26	-7	38	60	22	1.73	6.52	11.75	147	0.37	1.92	4.25	0.22	0.01	0.11	-3.28
Cntr	4	61	-7	43	71	28	1.54	4.68	11.21	163	0.42	1.58	2.72	0.25	0.01	0.11	0.9
LrD	1	1	-7	30	59	29	1.03	5.46	12.82	145	0.38	1.66	3.36	0.23	0.12	0.09	2.18

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Pcv	Neut	Lymph	Mono	Eosi	Baso	Pep
LrD	2	10	-7	36	63	27	1.33	5.7	11.24	159	0.4	1.36	3.94	0.25	0	0.11	1.55
LrD	2	14	-7	39	69	30	1.3	5.61	11.59	143	0.37	1.59	3.67	0.18	0.02	0.14	3.6
LrD	1	15	-7	40	64	24	1.67	6.4	13.21	167	0.44	1.38	4.38	0.43	0.07	0.12	3.78
LrD	2	24	-7	38	63	25	1.52	7.72	10.44	140	0.37	2.18	4.98	0.29	0.02	0.24	5.49
LrD	1	47	-7	36	59	23	1.57	4.01	10.22	145	0.39	0.77	2.91	0.2	0.01	0.12	-1.68
Cntr	4	3	1	37	63	26	1.62	5.96	11.67	161	0.41	1.84	3.76	0.15	0.01	0.19	-2.59
Cntr	3	11	1	38	63	25	1.56	4.85	10.71	153	0.39	1.74	2.82	0.14	0.01	0.12	1.5
Cntr	3	18	1	40	69	29	1.43	6.8	11.56	158	0.4	1.8	4.58	0.15	0.01	0.24	2.14
Cntr	4	21	1	40	64	24	1.65	10.78	11.03	149	0.39	3.12	7.2	0.25	0.02	0.18	2.59
Cntr	3	26	1	39	61	22	1.73	6.76	12.22	156	0.4	1.83	4.57	0.21	0.02	0.13	-6.13
Cntr	4	61	1	43	70	27	1.4	4.9	10.39	154	0.39	1.64	2.97	0.17	0.01	0.11	2.74
LrD	1	1	1	27	55	28	1.17	4.13	10.39	119	0.31	1.79	1.87	0.27	0.12	0.07	4.44
LrD	2	10	1	36	64	28	1.14	4.29	11.53	167	0.42	1.33	2.64	0.19	0.03	0.1	-1.94
LrD	2	14	1	38	69	31	1.26	4.55	11.2	137	0.36	1.49	2.78	0.12	0.04	0.1	4.14
LrD	1	15	1	38	64	26	1.28	4.92	13.07	170	0.44	1.21	3.32	0.2	0.06	0.12	3.34
LrD	2	24	1	37	63	26	1.55	6.44	10.55	142	0.37	1.91	4.06	0.25	0.06	0.16	2.19
LrD	1	47	1	37	61	24	1.56	2.79	10.07	140	0.38	0.63	1.92	0.13	0.02	0.08	0.82
Cntr	4	3	8	34	55	21	1.42	5.58	11.38	156	0.4	1.68	3.5	0.18	0.01	0.2	2.49
Cntr	3	11	8	39	64	25	1.52	4.15	10.26	144	0.38	1.28	2.51	0.19	0.01	0.15	-2.94
Cntr	3	18	8	40	68	28	1.38	5.39	11.05	148	0.38	1.46	3.58	0.16	0.01	0.17	1.3
Cntr	4	21	8	38	61	23	1.67	11.16	10.99	149	0.39	3.41	7.36	0.17	0.02	0.19	4.88
Cntr	3	26	8	38	60	22	1.77	6.25	11.67	148	0.38	1.78	4.13	0.22	0.01	0.11	-4.44
Cntr	4	61	8	42	72	30	1.59	3.71	10.96	168	0.43	1.08	2.37	0.16	0.01	0.1	-2.64
LrD	1	1	8	27	50	23	0.96	4.26	11.09	127	0.32	1.4	2.48	0.15	0.09	0.14	1.05
LrD	2	10	8	32	60	28	1.29	5.19	11.35	162	0.41	1.4	3.32	0.24	0.13	0.09	9.07
LrD	2	14	8	39	70	31	1.23	5.83	12.33	160	0.4	1.73	3.46	0.17	0.21	0.25	-4.24
LrD	1	15	8	37	66	29	1.46	6.18	12.63	165	0.43	0.94	4.83	0.18	0.22	0.38	2.84

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Pcv	Neut	Lymph	Mono	Eosi	Baso	Pep
LrD	2	24	8	34	56	22	1.42	7.25	10.42	142	0.37	1.99	4.32	0.48	0.16	0.26	4.83
LrD	1	47	8	39	64	25	1.54	3.89	9.77	137	0.36	0.68	2.69	0.21	0.11	0.18	-4.59
Cntr	4	3	15	37	63	26	1.42	6.01	11.55	170	0.41	1.77	3.69	0.38	0.03	0.15	1.23
Cntr	3	11	15	38	64	26	1.46	4.42	10.14	146	0.38	1.34	2.71	0.25	0.01	0.11	-6.3
Cntr	3	18	15	39	70	31	1.26	7.13	11.17	156	0.39	2.54	3.97	0.42	0.01	0.16	-0.05
Cntr	4	21	15	41	64	23	1.78	9.24	10.8	154	0.38	2.49	6.2	0.4	0.02	0.13	3.37
Cntr	3	26	15	38	62	24	1.58	5.96	11.42	148	0.38	1.82	3.86	0.17	0.02	0.09	-0.14
Cntr	4	61	15	43	71	28	1.54	3.93	10.72	172	0.43	1.29	2.36	0.15	0.01	0.11	3.51
LrD	1	1	15	27	58	31	0.87	3.68	11.1	132	0.33	1.03	2.19	0.21	0.11	0.13	9.81
LrD	2	10	15	34	67	33	1.03	5.07	10.79	164	0.4	1.2	3.35	0.14	0.29	0.07	-7.2
LrD	2	14	15	39	79	40	0.98	5.71	11.42	148	0.37	1.49	3.49	0.1	0.38	0.22	-4.46
LrD	1	15	15	37	68	31	1.19	6.14	12.27	164	0.41	1.09	4.25	0.2	0.34	0.26	0.85
LrD	2	24	15	34	64	30	1.13	8.36	10.03	141	0.36	1.92	5.11	0.45	0.51	0.36	-0.09
LrD	1	47	15	38	66	28	1.36	4.33	9.91	145	0.37	0.81	2.44	0.25	0.61	0.2	5.81
Cntr	4	3	22	41	64	23	1.78	5.04	11.89	176	0.43	1.59	3.13	0.14	0.02	0.15	0.42
Cntr	3	11	22	41	65	24	1.71	4.38	11.43	169	0.43	1.2	2.93	0.09	0.05	0.1	0.14
Cntr	3	18	22	40	69	29	1.38	7.57	11.95	170	0.42	3.09	3.53	0.73	0.01	0.18	0.89
Cntr	4	21	22	43	64	21	2.05	7.09	10.56	149	0.37	2.72	3.63	0.56	0.02	0.12	0.37
Cntr	3	26	22	39	60	21	1.86	6.67	11.87	159	0.4	1.87	4.51	0.15	0.04	0.11	1.82
Cntr	4	61	22	44	68	24	1.83	4.31	10.38	165	0.41	1.32	2.6	0.27	0.01	0.1	8.11
LrD	1	1	22	28	56	28	1	4.89	10.95	129	0.33	2.11	2.33	0.19	0.07	0.19	0.19
LrD	2	10	22	36	65	29	1.24	5.18	10.48	155	0.38	1.05	3.5	0.23	0.28	0.11	-0.37
LrD	2	14	22	38	72	34	1.12	6.46	10.39	137	0.34	1.5	4.04	0.22	0.7	0.32	-2.14
LrD	1	15	22	37	68	31	1.19	5.9	11.86	160	0.4	1.01	4.23	0.19	0.24	0.24	-4.1
LrD	2	24	22	36	64	28	1.29	7.11	9.79	143	0.36	1.75	4.48	0.2	0.39	0.28	1.35
LrD	1	47	22	41	66	25	1.64	4.07	9.59	138	0.35	0.7	2.96	0.13	0.28	0.25	13.37
Cntr	4	3	29	41	64	23	1.78	5.4	11.76	176	0.43	1.77	3.27	0.15	0.03	0.16	1.27

Treat	Pen	Anim	Day	Alb	TP	Glob	Agr	Wbc	Rbc	Hgb	Pcv	Neut	Lymph	Mono	Eosi	Baso	Pep
Cntr	3	11	29	39	62	23	1.7	4.38	9.93	148	0.37	1.09	3.04	0.14	0.01	0.11	3.75
Cntr	3	18	29	37	67	30	1.23	8.09	10.5	152	0.37	3.43	4.04	0.37	0.05	0.2	1.74
Cntr	4	21	29	42	64	22	1.91	9.25	10.6	154	0.38	2.59	5.88	0.6	0.02	0.15	7.19
Cntr	3	26	29	38	59	21	1.81	6.46	11.29	157	0.38	2.21	3.84	0.3	0.01	0.1	6.98
Cntr	4	61	29	44	71	27	1.63	3.87	10.27	170	0.41	1	2.54	0.18	0.03	0.12	3.27
LrD	1	1	29	26	55	29	0.9	3.7	9.95	116	0.29	1.44	1.78	0.24	0.08	0.16	2.75
LrD	2	10	29	34	65	31	1.1	5.45	10.55	159	0.39	1.08	3.97	0.16	0.14	0.1	4.91
LrD	2	14	29	37	75	38	0.97	5.93	11.09	148	0.36	1.27	3.72	0.16	0.5	0.26	4.02
LrD	1	15	29	37	71	34	1.09	6.28	11.08	153	0.38	0.88	4.67	0.22	0.21	0.29	4.91
LrD	2	24	29	34	64	30	1.13	6.53	9.12	133	0.33	1.6	4.18	0.25	0.2	0.3	4.76
LrD	1	47	29	40	66	26	1.54	3.65	10.96	160	0.4	0.58	2.85	0.16	0.06	0.23	6.53
Cntr	4	3	36	41	66	25	1.64	6.07	11.39	174	0.41	2.78	2.53	0.55	0.01	0.15	
Cntr	3	11	36	40	64	24	1.67	4.73	10.31	158	0.39	1.35	3.09	0.18	0.01	0.1	
Cntr	3	18	36	36	68	32	1.13	3.94	10.62	155	0.38	0.99	2.29	0.5	0.01	0.13	
Cntr	4	21	36	39	68	29	1.34	8.36	10.11	149	0.36	3.5	3.9	0.76	0.02	0.12	
Cntr	3	26	36	39	61	22	1.77	7.36	11.67	161	0.39	2.43	4.54	0.25	0.01	0.11	
Cntr	4	61	36	40	73	33	1.21	4.61	10.45	177	0.43	1.37	2.16	0.88	0.01	0.1	
LrD	1	1	36	24	51	27	0.89	4.27	11.14	134	0.34	1.66	2.19	0.15	0.16	0.11	
LrD	2	10	36	34	64	30	1.13	4.47	10.11	158	0.37	0.89	3.28	0.11	0.13	0.06	
LrD	2	14	36	36	71	35	1.03	5.65	10.12	134	0.33	1.4	3.72	0.09	0.25	0.18	
LrD	1	15	36	39	72	33	1.18	6.76	11.41	165	0.4	1.64	4.58	0.29	0.09	0.15	
LrD	2	24	36	32	66	34	0.94	8.66	9.1	137	0.34	2.3	4.85	1.19	0.02	0.23	
LrD	1	47	36	44	71	27	1.63	4.17	11.28	163	0.41	0.69	3.15	0.13	0.02	0.17	

1.2 Data- Faecal egg counts and Liveweight

Treat= Treatment group, LrD= Lower dose, Cntr= Control, Pen= Allocation of the deer on the pen, Anim= Identification number of the deer, Day= time before (-7) and after

(1-36) beginning of trickle infection, FEC= Faecal egg count (eggs per gram), LW= Live weight (Kg).

Treat	Pen	Anim	Day	LW	FEC
Cntr	4	3	-7	43	0
Cntr	3	11	-7	46.5	0
Cntr	3	18	-7	54.5	0
Cntr	4	21	-7	45.5	0
Cntr	3	26	-7	55.5	0
Cntr	4	61	-7	42	0
LrD	1	1	-7	29	0
LrD	2	10	-7	56	0
LrD	2	14	-7	52	0
LrD	1	15	-7	45	0
LrD	2	24	-7	58.5	0
LrD	1	47	-7	34	0
Cntr	4	3	1	44.5	0
Cntr	3	11	1	47.5	0
Cntr	3	18	1	55.5	0
Cntr	4	21	1	47	0
Cntr	3	26	1	56.5	0
Cntr	4	61	1	43	0
LrD	1	1	1	29	0
LrD	2	10	1	56.5	0
LrD	2	14	1	53.5	0
LrD	1	15	1	46.5	0
LrD	2	24	1	59.5	0
LrD	1	47	1	34.5	0
Cntr	4	3	8	45.5	0
Cntr	3	11	8	48	0

Treat	Pen	Anim	Day	LW	FEC
Cntr	3	18	8	55	0
Cntr	4	21	8	48.5	0
Cntr	3	26	8	56	0
Cntr	4	61	8	43.5	0
LrD	1	1	8	29	0
LrD	2	10	8	57	0
LrD	2	14	8	54.5	0
LrD	1	15	8	47.5	0
LrD	2	24	8	60	0
LrD	1	47	8	35	0
Cntr	4	3	15	45.5	0
Cntr	3	11	15	49	0
Cntr	3	18	15	57	0
Cntr	4	21	15	48.5	0
Cntr	3	26	15	57.5	0
Cntr	4	61	15	44	0
LrD	1	1	15	28.5	0
LrD	2	10	15	58	0
LrD	2	14	15	54.5	0
LrD	1	15	15	48	0
LrD	2	24	15	61	0
LrD	1	47	15	36	0
Cntr	4	3	22	46.5	0
Cntr	3	11	22	50	0
Cntr	3	18	22	57.5	0
Cntr	4	21	22	50	0
Cntr	3	26	22	59	0
Cntr	4	61	22	43.5	0
LrD	1	1	22	28.5	100

Treat	Pen	Anim	Day	LW	FEC
LrD	2	10	22	58	0
LrD	2	14	22	55	0
LrD	1	15	22	47.5	0
LrD	2	24	22	62.5	0
LrD	1	47	22	36.5	0
Cntr	4	3	29	48	0
Cntr	3	11	29	50.5	0
Cntr	3	18	29	58	0
Cntr	4	21	29	50	0
Cntr	3	26	29	59.5	0
Cntr	4	61	29	46	0
LrD	1	1	29	28.5	0
LrD	2	10	29	59	0
LrD	2	14	29	55.5	0
LrD	1	15	29	48.5	0
LrD	2	24	29	62.5	0
LrD	1	47	29	37.5	50
Cntr	4	3	36	49.5	0
Cntr	3	11	36	51.5	0
Cntr	3	18	36	58.5	0
Cntr	4	21	36	51	0
Cntr	3	26	36	60	0
Cntr	4	61	36	46	0
LrD	1	1	36	28.5	250
LrD	2	10	36	60.5	50
LrD	2	14	36	56.5	50
LrD	1	15	36	49.5	0
LrD	2	24	36	62.5	150
LrD	1	47	36	38	100

1.3 Data- Feed intake

Treat= Treatment group, LrD= Lower dose, Cntr= Control, Pen= Allocation of the deer on the pen, Anim= Identification number of the deer, Day n= individual intake (kg/head/day) of the group of deer in the pen, before (Day 0) and after (Day 1-42) beginning of trickle infection.

Treat	Pen	Anim	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Lrd	1	1,15,47	0.956	1.055	1.052	1.019	0.928	0.962	0.962	0.889	1.001	1.009	1.022	0.994	0.978	0.999	1.003
Lrd	2	10,74,24	1.422	1.478	1.53	1.492	1.413	1.425	1.394	1.376	1.45	1.428	1.31	1.452	1.324	1.38	1.345
Cntr	3	11,18,26	1.51	1.545	1.611	1.58	1.573	1.566	1.531	1.573	1.567	1.576	1.583	1.578	1.538	1.549	1.458
Cntr	4	3,21,61	1.266	1.373	1.37	1.368	1.39	1.38	1.403	1.39	1.424	1.436	1.433	1.411	1.365	1.405	1.333

Treat	Pen	Anim	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Lrd	1	1,15,47	0.986	0.913	0	0.938	1.014	1.039	0.965	1.018	1.104	1.117	1.134	1.115	1.067	1.047
Lrd	2	10,74,24	1.394	1.268	1.325	1.333	1.441	1.422	1.076	1.377	1.434	1.409	1.407	1.423	1.406	1.21
Cntr	3	11,18,26	1.504	1.349	1.508	1.461	1.602	1.578	1.236	1.52	1.592	1.607	1.562	1.594	1.57	1.394
Cntr	4	3,21,61	1.362	1.3	1.439	1.264	1.441	1.406	1.327	1.38	1.415	1.423	1.392	1.389	1.347	1.365

Treat	Pen	Anim	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42
Lrd	1	1,15,47	1.076	1.04	1.087	1.035	0.98	1.117	0.93	1.145	1.059	1.05	1.069	1.045	1.028	0.948
Lrd	2	10,74,24	1.326	1.421	1.426	1.334	1.367	1.496	1.352	1.415	1.265	1.374	1.355	1.375	1.465	1.35
Cntr	3	11,18,26	1.493	1.431	1.489	1.325	1.303	1.573	1.273	1.446	1.401	1.478	1.448	1.536	1.546	1.341
Cntr	4	3,21,61	1.312	1.401	1.435	1.345	1.276	1.49	1.282	1.289	1.336	1.305	1.36	1.311	1.361	1.228

1.4 Data- Nematode counts by genus and species

Treat= Treatment group, LrD= Lower dose, Anim= Identification number of the deer, Oster= *Ostertagia*-type nematode, Spi= *Spiculopteragia spiculoptera,* Asy= *Spiculopteragia asymmetrica,* Lep= *Ostertagia leptospicularis*, Kol= *Ostertagia kolchida*, Circ= *Teladorsagia circumcincta*, Haem= *Haemonchus contortus*, Trich Abo= *Trichostrongylus* spp. abomasum, Larvae Abo= Larvae from the abomasum, Oeso= *Oesophagostomum* spp., Oven= *Oesophagostomum venulosum*, Osik= *Oesophagostomum sikae*, Trich SI= *Trichostrongylus* spp. from the small intestine, Coop= *Cooperia* spp.

Anim	Treat	Oster	Spi	Asy	Lep	Kol	Circ	Haem	Trich Abo	Larvae Abo	Oeso	Oven	Osik	Trich SI	Coop
1	LrD	280	56	112	112	0	0	0	0	40	400	343	114	0	0
10	LrD	240	0	240	0	0	0	20	0	0	160	160	0	0	0
14	LrD	100	.	.				0	0	0	160	160	0	0	0
15	LrD	80	0	40	40	0	0	0	0	0	180	180	0	0	0
24	LrD	420	23	140	47	0	0	0	0	40	220	220	0	0	0
47	LrD	160	40	120	0	0	0	0	0	0	400	338	62	0	0

Appendix 4 Supplementary information for Chapter 4. Identification and distribution of gastrointestinal nematodes on red deer in New Zealand

4.1 Data- Identification of larvae spp. by farm, island, region, animal host spp. and season

Anim host spp.= Animal host species, DS= farms that graze deer and sheep, DC= farm that graze deer and cattle, D= farms that only have deer, DSC= farm that graze deer, sheep and cattle, GIN spp.= Gastrointestinal nematode species.

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*S. asymmetrica*	Spring
J158	south	Canterbury	DS	*S. asymmetrica*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*S. asymmetrica*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS	*O. venulosum*	Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS		Spring
J158	south	Canterbury	DS	*S. asymmetrica*	Spring
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC		Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC		Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC		Winter
J005	north	Manawatu-Wanganui	DSC		Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC		Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J005	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J005	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*T. askivali*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*O. venulosum*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC		Winter
J011	north	Manawatu-Wanganui	DC		Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC		Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. askivali*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J011	north	Manawatu-Wanganui	DC	*T. axei*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D		Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D	*O. sikae*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J021	north	Manawatu-Wanganui	D		Winter
J021	north	Manawatu-Wanganui	D		Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D		Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J021	north	Manawatu-Wanganui	D	*S. asymmetrica*	Winter
J021	north	Manawatu-Wanganui	D	*S. asymmetrica*	Winter
J021	north	Manawatu-Wanganui	D		Winter
J021	north	Manawatu-Wanganui	D	*S. asymmetrica*	Winter
J021	north	Manawatu-Wanganui	D	*S. asymmetrica*	Winter
J021	north	Manawatu-Wanganui	D	*C. oncophora*	Winter
J021	north	Manawatu-Wanganui	D		Winter
J021	north	Manawatu-Wanganui	D	*S. asymmetrica*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J021	north	Manawatu-Wanganui	D	*O. leptospicularis*	Winter
J030	north	Waikato	DSC	*T. askivali*	Winter
J030	north	Waikato	DSC	*C. oncophora*	Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC	*T. askivali*	Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC	*C. oncophora*	Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC	*C. oncophora*	Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC		Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC	*C. oncophora*	Winter
J030	north	Waikato	DSC	*T. vitrinus*	Winter
J030	north	Waikato	DSC	*T. vitrinus*	Winter
J030	north	Waikato	DSC	*T. askivali*	Winter
J030	north	Waikato	DSC	*T. vitrinus*	Winter
J030	north	Waikato	DSC		Winter
J030	north	Waikato	DSC	*T. vitrinus*	Winter
J030	north	Waikato	DSC	*T. vitrinus*	Winter
J030	north	Waikato	DSC	*T. vitrinus*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*T. askivali*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D		Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D		Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J036	north	Manawatu-Wanganui	D	*O. venulosum*	Winter
J037	north	Manawatu-Wanganui	DSC	*T. askivali*	Winter
J037	north	Manawatu-Wanganui	DSC	*O. leptospicularis*	Winter
J037	north	Manawatu-Wanganui	DSC	*C. oncophora*	Winter
J037	north	Manawatu-Wanganui	DSC	*T. askivali*	Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC	*O. leptospicularis*	Winter
J037	north	Manawatu-Wanganui	DSC	*O. leptospicularis*	Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J037	north	Manawatu-Wanganui	DSC	*O. leptospicularis*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC	*O. leptospicularis*	Winter
J037	north	Manawatu-Wanganui	DSC	*T. colubriformis*	Winter
J037	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J037	north	Manawatu-Wanganui	DSC	*T. axei*	Winter
J037	north	Manawatu-Wanganui	DSC	*O. leptospicularis*	Winter
J037	north	Manawatu-Wanganui	DSC	*T. circumcincta*	Winter
J037	north	Manawatu-Wanganui	DSC		Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*S. asymmetrica*	Winter
J046	north	Hawkes Bay	DC	*C. oncophora*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC		Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC		Winter
J046	north	Hawkes Bay	DC	*C. oncophora*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*C. oncophora*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J046	north	Hawkes Bay	DC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC		Winter
J062	north	Manawatu-Wanganui	DSC	*T. vitrinus*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J062	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. spiculoptera*	Winter
J074	north	Manawatu-Wanganui	DSC	*T. askivali*	Winter
J074	north	Manawatu-Wanganui	DSC	*T. vitrinus*	Winter
J074	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. spiculoptera*	Winter
J074	north	Manawatu-Wanganui	DSC	*T. vitrinus*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. spiculoptera*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J074	north	Manawatu-Wanganui	DSC	*T. axei*	Winter
J074	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC		Winter
J074	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC	*T. axei*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J074	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J074	north	Manawatu-Wanganui	DSC	*T. axei*	Winter
J092	north	Manawatu-Wanganui	DS	*T. axei*	Winter
J092	north	Manawatu-Wanganui	DS	*T. axei*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*S. asymmetrica*	Winter
J092	north	Manawatu-Wanganui	DS	*S. asymmetrica*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*S. asymmetrica*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*S. asymmetrica*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*C. oncophora*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J092	north	Manawatu-Wanganui	DS	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*T. axei*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*T. axei*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*S. spiculoptera*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*S. spiculoptera*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*S. spiculoptera*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*S. asymmetrica*	Winter
J100	north	Hawkes Bay	DC	*T. axei*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*T. axei*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*S. spiculoptera*	Winter
J100	north	Hawkes Bay	DC	*S. spiculoptera*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J100	north	Hawkes Bay	DC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. spiculoptera*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC		Winter
J114	north	Manawatu-Wanganui	DSC	*T. colubriformis*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. spiculoptera*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC		Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J114	north	Manawatu-Wanganui	DSC	*O. venulosum*	Winter
J114	north	Manawatu-Wanganui	DSC	*S. asymmetrica*	Winter
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*T. axei*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*T. axei*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*T. axei*	Spring
J167	north	Hawkes Bay	DSC	*O. leptospicularis*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*O. leptospicularis*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*S. asymmetrica*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*T. vitrinus*	Spring
J167	north	Hawkes Bay	DSC	*S. spiculoptera*	Spring
J167	north	Hawkes Bay	DSC	*O. leptospicularis*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*T. circumcincta*	Spring
J168	north	Hawkes Bay	D		Spring
J168	north	Hawkes Bay	D	*C. oncophora*	Spring
J168	north	Hawkes Bay	D		Spring
J168	north	Hawkes Bay	D	*S. asymmetrica*	Spring
J168	north	Hawkes Bay	D	*O. venulosum*	Spring
J168	north	Hawkes Bay	D	*C. oncophora*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*C. oncophora*	Spring
J168	north	Hawkes Bay	D	*O. venulosum*	Spring
J168	north	Hawkes Bay	D	*O. venulosum*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*T. vitrinus*	Spring
J168	north	Hawkes Bay	D	*O. venulosum*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J168	north	Hawkes Bay	D	*S. spiculoptera*	Spring
J183	north	Manawatu-Wanganui	DC	*T. circumcincta*	Spring
J183	north	Manawatu-Wanganui	DC		Spring
J183	north	Manawatu-Wanganui	DC	*O. leptospicularis*	Spring
J183	north	Manawatu-Wanganui	DC	*O. venulosum*	Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring
J183	north	Manawatu-Wanganui	DC	*O. leptospicularis*	Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring
J183	north	Manawatu-Wanganui	DC	*O. venulosum*	Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring
J183	north	Manawatu-Wanganui	DC	*O. venulosum*	Spring
J183	north	Manawatu-Wanganui	DC	*O. venulosum*	Spring
J183	north	Manawatu-Wanganui	DC	*T. vitrinus*	Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J183	north	Manawatu-Wanganui	DC		Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring
J183	north	Manawatu-Wanganui	DC	*O. venulosum*	Spring
J183	north	Manawatu-Wanganui	DC	*S. spiculoptera*	Spring
J183	north	Manawatu-Wanganui	DC	*O. venulosum*	Spring
J183	north	Manawatu-Wanganui	DC	*T. askivali*	Spring
J183	north	Manawatu-Wanganui	DC	*T. askivali*	Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring
J183	north	Manawatu-Wanganui	DC	*T. circumcincta*	Spring
J183	north	Manawatu-Wanganui	DC		Spring
J183	north	Manawatu-Wanganui	DC	*S. asymmetrica*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*T. askivali*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*T. axei*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*T. vitrinus*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC		Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J204	north	East Coast	DSC	*O. venulosum*	Spring
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*S. asymmetrica*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*S. asymmetrica*	Winter
J008	south	Canterbury	D	*S. asymmetrica*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*S. asymmetrica*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J008	south	Canterbury	D	*S. asymmetrica*	Winter
J008	south	Canterbury	D	*O. venulosum*	Winter
J014	south	Southland	DS	*S. asymmetrica*	Winter
J014	south	Southland	DS	*S. asymmetrica*	Winter
J014	south	Southland	DS	*T. askivali*	Winter
J014	south	Southland	DS		Winter
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS	*T. colubriformis*	Winter
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS	*O. venulosum*	Winter
J014	south	Southland	DS	*O. leptospicularis*	Winter
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS		Winter
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS	*O. venulosum*	Winter
J014	south	Southland	DS	*O. leptospicularis*	Winter
J014	south	Southland	DS	*T. colubriformis*	Winter
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS	*S. asymmetrica*	Winter
J014	south	Southland	DS	*S. spiculoptera*	Winter
J014	south	Southland	DS	*O. venulosum*	Winter
J014	south	Southland	DS	*O. leptospicularis*	Winter
J014	south	Southland	DS	*T. askivali*	Winter
J014	south	Southland	DS		Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*S. asymmetrica*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*S. asymmetrica*	Winter
J068	south	Canterbury	D	*S. asymmetrica*	Winter
J068	south	Canterbury	D	*O. venulosum*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*S. asymmetrica*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*S. asymmetrica*	Winter
J068	south	Canterbury	D	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J068	south	Canterbury	D	*O. venulosum*	Winter
J068	south	Canterbury	D	*O. venulosum*	Winter
J068	south	Canterbury	D	*O. venulosum*	Winter
J068	south	Canterbury	D	*S. asymmetrica*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. leptospicularis*	Winter
J068	south	Canterbury	D	*O. sikae*	Winter
J073	south	Southland	D		Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*S. asymmetrica*	Winter
J073	south	Southland	D	*T. colubriformis*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*S. asymmetrica*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D		Winter
J073	south	Southland	D	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*S. asymmetrica*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J073	south	Southland	D	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*C. oncophora*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*T. askivali*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*T. askivali*	Winter
J097	south	Southland	DS	*S. spiculoptera*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS		Winter
J097	south	Southland	DS	*S. asymmetrica*	Winter
J097	south	Southland	DS	*S. spiculoptera*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS		Winter
J097	south	Southland	DS	*T. askivali*	Winter
J097	south	Southland	DS	*T. askivali*	Winter
J097	south	Southland	DS	*S. asymmetrica*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J097	south	Southland	DS	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC		Winter
J113	south	Canterbury	DSC	*S. spiculoptera*	Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*O. sikae*	Winter
J113	south	Canterbury	DSC	*T. vitrinus*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*S. spiculoptera*	Winter
J113	south	Canterbury	DSC	*O. leptospicularis*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J113	south	Canterbury	DSC		Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC		Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*O. venulosum*	Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J113	south	Canterbury	DSC	*T. askivali*	Winter
J117	south	Canterbury	D	*T. colubriformis*	Winter
J117	south	Canterbury	D	*C. oncophora*	Winter
J117	south	Canterbury	D		Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter
J117	south	Canterbury	D	*T. colubriformis*	Winter
J117	south	Canterbury	D	*T. circumcincta*	Winter
J117	south	Canterbury	D	*T. vitrinus*	Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter
J117	south	Canterbury	D	*S. spiculoptera*	Winter
J117	south	Canterbury	D	*O. venulosum*	Winter
J117	south	Canterbury	D		Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J117	south	Canterbury	D	*T. vitrinus*	Winter
J117	south	Canterbury	D	*T. colubriformis*	Winter
J117	south	Canterbury	D	*S. asymmetrica*	Winter
J117	south	Canterbury	D	*T. colubriformis*	Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter
J117	south	Canterbury	D	*S. spiculoptera*	Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter
J117	south	Canterbury	D	*T. circumcincta*	Winter
J117	south	Canterbury	D	*T. colubriformis*	Winter
J117	south	Canterbury	D	*O. leptospicularis*	Winter
J117	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*S. asymmetrica*	Winter
J119	south	Canterbury	D	*T. axei*	Winter
J119	south	Canterbury	D	*T. axei*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*T. axei*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*T. axei*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*S. spiculoptera*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J119	south	Canterbury	D		Winter
J119	south	Canterbury	D	*O. venulosum*	Winter
J122	south	Otago	DS	*C. oncophora*	Winter
J122	south	Otago	DS	*S. asymmetrica*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*C. oncophora*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. leptospicularis*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*T. colubriformis*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. leptospicularis*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J122	south	Otago	DS	*O. venulosum*	Winter
J123	south	Otago	DSC		Winter
J123	south	Otago	DSC	*O. leptospicularis*	Winter
J123	south	Otago	DSC	*S. spiculoptera*	Winter
J123	south	Otago	DSC	*O. sikae*	Winter
J123	south	Otago	DSC	*C. oncophora*	Winter
J123	south	Otago	DSC	*S. spiculoptera*	Winter
J123	south	Otago	DSC	*T. askivali*	Winter
J123	south	Otago	DSC	*O. leptospicularis*	Winter
J123	south	Otago	DSC	*S. asymmetrica*	Winter
J123	south	Otago	DSC	*S. spiculoptera*	Winter
J123	south	Otago	DSC	*H. contortus*	Winter
J123	south	Otago	DSC	*T. askivali*	Winter
J123	south	Otago	DSC	*O. leptospicularis*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J123	south	Otago	DSC	*O. leptospicularis*	Winter
J123	south	Otago	DSC	*O. leptospicularis*	Winter
J123	south	Otago	DSC	*S. asymmetrica*	Winter
J123	south	Otago	DSC	*S. asymmetrica*	Winter
J123	south	Otago	DSC	*T. colubriformis*	Winter
J123	south	Otago	DSC	*S. spiculoptera*	Winter
J123	south	Otago	DSC	*C. oncophora*	Winter
J123	south	Otago	DSC	*O. leptospicularis*	Winter
J123	south	Otago	DSC	*T. colubriformis*	Winter
J123	south	Otago	DSC	*O. leptospicularis*	Winter
J123	south	Otago	DSC	*T. axei*	Winter
J130	south	Canterbury	DC	*S. spiculoptera*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*S. spiculoptera*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J130	south	Canterbury	DC	*S. spiculoptera*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J130	south	Canterbury	DC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*S. asymmetrica*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. leptospicularis*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*S. asymmetrica*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*O. venulosum*	Winter
J131	south	Canterbury	DSC	*S. asymmetrica*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*T. axei*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*C. oncophora*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J135	south	Otago	DS	*O. venulosum*	Winter
J214	south	Canterbury	DSC	*S. spiculoptera*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*S. asymmetrica*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*T. circumcincta*	Spring
J214	south	Canterbury	DSC	*T. circumcincta*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*S. asymmetrica*	Spring
J214	south	Canterbury	DSC	*S. spiculoptera*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*S. spiculoptera*	Spring
J214	south	Canterbury	DSC	*S. asymmetrica*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*S. spiculoptera*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*O. leptospicularis*	Spring
J214	south	Canterbury	DSC	*S. spiculoptera*	Spring
J214	south	Canterbury	DSC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*O. venulosum*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*T. circumcincta*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*O. leptospicularis*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J165	south	Canterbury	DC	*T. circumcincta*	Spring
J165	south	Canterbury	DC	*S. asymmetrica*	Spring
J176	south	Canterbury	DC	*O. venulosum*	Spring
J176	south	Canterbury	DC	*T. circumcincta*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*C. oncophora*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC		Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*S. asymmetrica*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*C. oncophora*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC		Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J176	south	Canterbury	DC	*H. contortus*	Spring
J180	south	Southland	DC	*T. axei*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*T. axei*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*T. colubriformis*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*T. axei*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*T. axei*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*S. asymmetrica*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*O. venulosum*	Spring
J180	south	Southland	DC	*S. asymmetrica*	Spring
J190	south	Canterbury	D		Spring
J190	south	Canterbury	D	*S. spiculoptera*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*O. leptospicularis*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*T. circumcincta*	Spring
J190	south	Canterbury	D	*T. circumcincta*	Spring
J190	south	Canterbury	D	*H. contortus*	Spring
J190	south	Canterbury	D	*S. spiculoptera*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. spiculoptera*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J190	south	Canterbury	D	*S. spiculoptera*	Spring
J190	south	Canterbury	D	*T. askivali*	Spring
J190	south	Canterbury	D	*H. contortus*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D		Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D	*S. asymmetrica*	Spring
J190	south	Canterbury	D		Spring
J194	south	Canterbury	DSC	*S. spiculoptera*	Spring
J194	south	Canterbury	DSC	*T. circumcincta*	Spring
J194	south	Canterbury	DSC		Spring
J194	south	Canterbury	DSC	*T. vitrinus*	Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC	*O. leptospicularis*	Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC		Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC	*O. leptospicularis*	Spring
J194	south	Canterbury	DSC	*T. vitrinus*	Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J194	south	Canterbury	DSC		Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC	*S. spiculoptera*	Spring
J194	south	Canterbury	DSC		Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC	*T. circumcincta*	Spring
J194	south	Canterbury	DSC	*H. contortus*	Spring
J194	south	Canterbury	DSC	*T. askivali*	Spring
J194	south	Canterbury	DSC	*T. askivali*	Spring
J194	south	Canterbury	DSC	*T. askivali*	Spring
J194	south	Canterbury	DSC	*T. askivali*	Spring
J211	south	Canterbury	D	*O. leptospicularis*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*O. leptospicularis*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*O. leptospicularis*	Spring
J211	south	Canterbury	D	*T. askivali*	Spring
J211	south	Canterbury	D	*O. leptospicularis*	Spring
J211	south	Canterbury	D		Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. asymmetrica*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. spiculoptera*	Spring
J211	south	Canterbury	D	*S. asymmetrica*	Spring
J211	south	Canterbury	D	*O. leptospicularis*	Spring
J211	south	Canterbury	D	*O. venulosum*	Spring
J211	south	Canterbury	D	*C. oncophora*	Spring
J211	south	Canterbury	D	*O. sikae*	Spring
J211	south	Canterbury	D	*O. sikae*	Spring
J215	south	Canterbury	DSC		Spring
J215	south	Canterbury	DSC	*S. spiculoptera*	Spring
J215	south	Canterbury	DSC	*S. spiculoptera*	Spring
J215	south	Canterbury	DSC	*S. spiculoptera*	Spring
J215	south	Canterbury	DSC	*O. leptospicularis*	Spring
J215	south	Canterbury	DSC	*O. sikae*	Spring
J215	south	Canterbury	DSC	*T. circumcincta*	Spring
J215	south	Canterbury	DSC	*O. leptospicularis*	Spring
J215	south	Canterbury	DSC	*O. sikae*	Spring
J215	south	Canterbury	DSC		Spring
J215	south	Canterbury	DSC	*O. leptospicularis*	Spring
J215	south	Canterbury	DSC	*T. circumcincta*	Spring
J215	south	Canterbury	DSC	*O. venulosum*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J215	south	Canterbury	DSC	*O. leptospicularis*	Spring
J215	south	Canterbury	DSC	*S. spiculoptera*	Spring
J215	south	Canterbury	DSC	*O. sikae*	Spring
J215	south	Canterbury	DSC	*O. sikae*	Spring
J215	south	Canterbury	DSC	*O. leptospicularis*	Spring
J215	south	Canterbury	DSC	*O. sikae*	Spring
J215	south	Canterbury	DSC	*O. venulosum*	Spring
J215	south	Canterbury	DSC	*O. sikae*	Spring
J215	south	Canterbury	DSC	*S. spiculoptera*	Spring
J215	south	Canterbury	DSC	*S. spiculoptera*	Spring
J215	south	Canterbury	DSC	*O. leptospicularis*	Spring
J195	north	Waikato	D		Spring
J195	north	Waikato	D		Spring
J195	north	Waikato	D	*O. venulosum*	Spring
J195	north	Waikato	D	*H. contortus*	Spring
J195	north	Waikato	D	*C. curticei*	Spring
J195	north	Waikato	D		Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J195	north	Waikato	D	*O. sikae*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D		Spring
J195	north	Waikato	D	*H. contortus*	Spring
J195	north	Waikato	D	*T. askivali*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J195	north	Waikato	D	*S. spiculoptera*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. spiculoptera*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*O. venulosum*	Spring
J217	south	Canterbury	DSC	*S. spiculoptera*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. spiculoptera*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*O. leptospicularis*	Spring
J217	south	Canterbury	DSC	*S. spiculoptera*	Spring
J217	south	Canterbury	DSC	*O. leptospicularis*	Spring
J217	south	Canterbury	DSC	*S. spiculoptera*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J217	south	Canterbury	DSC	*S. spiculoptera*	Spring
J217	south	Canterbury	DSC	*O. leptospicularis*	Spring
J217	south	Canterbury	DSC	*S. asymmetrica*	Spring
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*S. asymmetrica*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*S. spiculoptera*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC	*S. spiculoptera*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J244	north	Gisborne	DSC		Summer
J244	north	Gisborne	DSC	*C. oncophora*	Summer
J244	north	Gisborne	DSC	*O. venulosum*	Summer
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*T. vitrinus*	Autumm
J258	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J258	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J258	north	Manawatu-Wanganui	D		Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D		Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J258	north	Manawatu-Wanganui	D	*T. vitrinus*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D		Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J258	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D		Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J259	north	Manawatu-Wanganui	D	*H. contortus*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D		Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J259	north	Manawatu-Wanganui	D	*H. contortus*	Autumm
J259	north	Manawatu-Wanganui	D	*O. venulosum*	Autumm
J261	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J261	north	Manawatu-Wanganui	D		Autumm
J261	north	Manawatu-Wanganui	D		Autumm
J261	north	Manawatu-Wanganui	D	*H. contortus*	Autumm
J261	north	Manawatu-Wanganui	D	*O. leptospicularis*	Autumm
J261	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J261	north	Manawatu-Wanganui	D	*O. leptospicularis*	Autumm
J261	north	Manawatu-Wanganui	D	*O. sikae*	Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*T. circumcincta*	Autumm
J261	north	Manawatu-Wanganui	D	*T. vitrinus*	Autumm
J261	north	Manawatu-Wanganui	D		Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J261	north	Manawatu-Wanganui	D		Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J261	north	Manawatu-Wanganui	D	*T. vitrinus*	Autumm
J261	north	Manawatu-Wanganui	D	*S. asymmetrica*	Autumm
J261	north	Manawatu-Wanganui	D	*S. spiculoptera*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*S. asymmetrica*	Autumm
J264	south	Canterbury	D	*S. spiculoptera*	Autumm
J264	south	Canterbury	D	*T. circumcincta*	Autumm
J264	south	Canterbury	D	*T. askivali*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*S. asymmetrica*	Autumm
J264	south	Canterbury	D	*S. spiculoptera*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*S. asymmetrica*	Autumm
J264	south	Canterbury	D	*S. asymmetrica*	Autumm
J264	south	Canterbury	D		Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J264	south	Canterbury	D	*T. askivali*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*S. spiculoptera*	Autumm
J264	south	Canterbury	D	*S. spiculoptera*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*T. vitrinus*	Autumm
J264	south	Canterbury	D	*T. askivali*	Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J264	south	Canterbury	D	*O. leptospicularis*	Autumm
J264	south	Canterbury	D		Autumm
J264	south	Canterbury	D	*O. venulosum*	Autumm
J274	south	Canterbury	DC		Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC		Autumm
J274	south	Canterbury	DC	*S. spiculoptera*	Autumm
J274	south	Canterbury	DC		Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*S. spiculoptera*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*S. asymmetrica*	Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*S. spiculoptera*	Autumm
J274	south	Canterbury	DC	*T. circumcincta*	Autumm
J274	south	Canterbury	DC	*O. venulosum*	Autumm
J274	south	Canterbury	DC	*O. sikae*	Autumm
J274	south	Canterbury	DC	*S. asymmetrica*	Autumm
J274	south	Canterbury	DC		Autumm
J227	south	Canterbury	DSC	*T. vitrinus*	Summer
J227	south	Canterbury	DSC	*T. circumcincta*	Summer
J227	south	Canterbury	DSC	*S. spiculoptera*	Summer
J227	south	Canterbury	DSC	*T. vitrinus*	Summer
J227	south	Canterbury	DSC	*S. spiculoptera*	Summer
J227	south	Canterbury	DSC	*T. circumcincta*	Summer
J227	south	Canterbury	DSC	*O. leptospicularis*	Summer
J227	south	Canterbury	DSC	*S. spiculoptera*	Summer
J227	south	Canterbury	DSC	*T. vitrinus*	Summer
J227	south	Canterbury	DSC	*T. vitrinus*	Summer
J227	south	Canterbury	DSC	*S. spiculoptera*	Summer
J227	south	Canterbury	DSC		Summer
J227	south	Canterbury	DSC		Summer

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
J227	south	Canterbury	DSC	*T. vitrinus*	Summer
J227	south	Canterbury	DSC	*T. circumcincta*	Summer
J227	south	Canterbury	DSC		Summer
J227	south	Canterbury	DSC		Summer
J227	south	Canterbury	DSC	*O. leptospicularis*	Summer
J227	south	Canterbury	DSC	*O. venulosum*	Summer
J227	south	Canterbury	DSC		Summer
J227	south	Canterbury	DSC	*S. spiculoptera*	Summer
J227	south	Canterbury	DSC		Summer
J227	south	Canterbury	DSC		Summer
J227	south	Canterbury	DSC		Summer
L7	south	Southland	DSC	*O. sikae*	Autumm
L7	south	Southland	DSC	*T. colubriformis*	Autumm
L7	south	Southland	DSC	*O. venulosum*	Autumm
L7	south	Southland	DSC	*S. asymmetrica*	Autumm
L7	south	Southland	DSC	*S. spiculoptera*	Autumm
L7	south	Southland	DSC	*O. venulosum*	Autumm
L7	south	Southland	DSC		Autumm
L7	south	Southland	DSC	*T. vitrinus*	Autumm
L7	south	Southland	DSC		Autumm
L7	south	Southland	DSC	*T. circumcincta*	Autumm
L7	south	Southland	DSC	*O. sikae*	Autumm
L7	south	Southland	DSC	*O. sikae*	Autumm
L7	south	Southland	DSC	*T. colubriformis*	Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L7	south	Southland	DSC	*O. sikae*	Autumm
L7	south	Southland	DSC	*T. circumcincta*	Autumm
L7	south	Southland	DSC	*O. leptospicularis*	Autumm
L7	south	Southland	DSC	*S. asymmetrica*	Autumm
L7	south	Southland	DSC		Autumm
L7	south	Southland	DSC	*O. sikae*	Autumm
L7	south	Southland	DSC	*S. asymmetrica*	Autumm
L7	south	Southland	DSC	*O. venulosum*	Autumm
L7	south	Southland	DSC	*O. leptospicularis*	Autumm
L7	south	Southland	DSC	*C. oncophora*	Autumm
L7	south	Southland	DSC	*S. asymmetrica*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L13	north	Central Plateau	DSC	*C. oncophora*	Autumm
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC		Winter
L36	north	Hawkes Bay	DSC		Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC		Winter
L36	north	Hawkes Bay	DSC	*O. leptospicularis*	Winter
L36	north	Hawkes Bay	DSC	*O. leptospicularis*	Winter
L36	north	Hawkes Bay	DSC	*O. leptospicularis*	Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC	*O. leptospicularis*	Winter
L36	north	Hawkes Bay	DSC	*C. oncophora*	Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC	*O. leptospicularis*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L36	north	Hawkes Bay	DSC		Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC	*C. oncophora*	Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC	*O. venulosum*	Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC	*S. asymmetrica*	Winter
L36	north	Hawkes Bay	DSC		Winter
L36	north	Hawkes Bay	DSC		Winter
L36	north	Hawkes Bay	DSC	*C. oncophora*	Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC	*C. curticei*	Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC		Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC	*O. venulosum*	Winter
L45	north	Central Plateau	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L47	south	West Coast	DSC	*O. leptospicularis*	Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L47	south	West Coast	DSC	*O. leptospicularis*	Winter
L47	south	West Coast	DSC	*T. circumcincta*	Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC		Winter
L47	south	West Coast	DSC	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*S. asymmetrica*	Winter
L56	south	West Coast	DS		Winter
L56	south	West Coast	DS	*T. colubriformis*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*T. vitrinus*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS	*O. venulosum*	Winter
L56	south	West Coast	DS		Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC		Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*S. asymmetrica*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC		Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L66	south	Otago	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC		Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. leptospicularis*	Winter
L57	south	Southland	DSC	*T. colubriformis*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*T. vitrinus*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L57	south	Southland	DSC		Winter
L57	south	Southland	DSC		Winter
L57	south	Southland	DSC		Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC		Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC		Winter
L57	south	Southland	DSC	*O. venulosum*	Winter
L57	south	Southland	DSC	*T. askivali*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*S. asymmetrica*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D		Winter
L71	south	West Coast	D		Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D		Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D		Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D		Winter
L71	south	West Coast	D	*S. asymmetrica*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L71	south	West Coast	D	*T. askivali*	Winter
L71	south	West Coast	D	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC		Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC		Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC		Winter
L88	south	Southland	DSC	*O. venulosum*	Winter

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. leptospicularis*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*O. venulosum*	Winter
L88	south	Southland	DSC	*S. asymmetrica*	Winter
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC	*O. leptospicularis*	Spring
L113	south	West Coast	DC	*O. leptospicularis*	Spring
L113	south	West Coast	DC	*S. asymmetrica*	Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC	*T. askivali*	Spring
L113	south	West Coast	DC	*S. asymmetrica*	Spring
L113	south	West Coast	DC	*O. leptospicularis*	Spring
L113	south	West Coast	DC	*O. leptospicularis*	Spring
L113	south	West Coast	DC	*S. asymmetrica*	Spring
L113	south	West Coast	DC	*O. leptospicularis*	Spring
L113	south	West Coast	DC	*T. circumcincta*	Spring
L113	south	West Coast	DC		Spring
L113	south	West Coast	DC	*O. leptospicularis*	Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring

Farm Code	Island	Region	Anim host spp.	GIN spp.	Season
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC		Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC	*O. venulosum*	Spring
L98	north	Central Plateau	DSC	*O. leptospicularis*	Spring
L98	north	Central Plateau	DSC	*S. asymmetrica*	Spring

Appendix 5 Supplementary information for Chapter 5. Establishment rate of sheep gastrointestinal nematodes in farmed red deer (*Cervus elaphus*).

5.1. Data- Liveweight and Faecal egg counts

Anim= Identification number of the deer, FEC(n)= Faecal egg count (eggs per gram) on the day (1-28) from the infection dose, Weight(n)= Live weight (Kg) on the day (1-28) from the infection dose.

Anim	Species	Weight(1)	Fec(1)	Weight(7)	Fec(7)	Weight(14)	Fec(14)	Weight(21)	Fec(21)	Weight(28)	Fec(28)
117	deer	64	0	64	0	64.5	0	64	200	64.5	800
118	deer	60	0	62	0	63.5	0	64	50	64	450
120	deer	60	0	62	0	61.5	0	62.5	150	63	50
122	deer	58	0	60.5	0	60.5	0	61.5	50	61	200
123	deer	63	0	65	0	65.5	0	65.5	50	65	250
409	sheep	21	0	23	0	22	0	22.7	650	24	1450
416	sheep	27	0	20	0	31	0	31.7	1150	32.5	1000
417	sheep	23	0	26	0	27	0	26.4	2250	26.5	4950
418	sheep	34	0	37	0	36	0	36.8	1200	38	1200
420	sheep	19	0	22	0	23	0	22.8	1400	22	4750

5.2. Data- Individual dose of infective larvae

Anim= Identification number of the deer, *Trich* total= *Trichostrongylus spp.* infective larvae (L3) of the abomasum and Small intestine, *Coop*= *Cooperia spp.* L3, Telad= *Teladorsagia circumcincta* L3, Oeso/Chab= *Oesophagostomum venulosum* L3 and *Chabertia ovina* L3, Haem= *Haemonchus contortus* L3, Nemat= *Nematodirus* spp. L3.

Anim	Species	Dose (ml)	*Trich* total	*Coop*	*Telad*	*Oeso/Chab*	*Haem*	*Nemat*
117	deer	40.0	3000	2259	6000	6633	3000	38
118	deer	37.5	2812	2117	5625	6218	2812	36
120	deer	37.5	2812	2117	5625	6218	2812	36
122	deer	36.3	2719	2047	5438	6011	2719	35
123	deer	39.4	2953	2223	5906	6529	2953	38
409	sheep	13.1	984	741	1969	2176	984	13
416	sheep	16.9	1266	953	2531	2798	1266	16
417	sheep	14.4	1078	812	2156	2384	1078	14
418	sheep	21.3	1594	1200	3188	3524	1594	20
420	sheep	11.9	891	671	1781	1969	891	11

5.3. Data- Individual Nematode burdens.

Anim= Identification number of the deer, *T. vitr*= *Trichostrongylus vitrinus*, *T. colub*= *Trichostrongylus colubriformis, C. curt*= *Cooperia curticei,* Telad= *Teladorsagia circumcincta*, *Haem*= *Haemonchus contortus*, *Trich*= *Trichostrongylus axei,* Oeso= *Oesophagostomum venulosum*, Chab= *Chabertia ovina*.

		Small intestine			Abomasum			Large intestine	
Anim	Species	*T.vitr*	*T. colub*	*C.curt*	*Telad*	*Haem*	*Trich*	*Oeso*	*Chab*
117	deer	0	0	0	140	390	50	490	0
118	deer	0	0	0	30	260	20	40	0
120	deer	0	0	0	0	180	20	360	0
122	deer	0	0	0	70	320	40	470	0
123	deer	0	0	40	160	380	20	320	0
409	sheep	324	216	200	560	180	80	440	70
416	sheep	543	78	260	620	140	120	600	40
417	sheep	715	55	350	1110	250	70	860	10
418	sheep	947	473	230	1070	340	20	80	0
420	sheep	440	240	260	730	180	30	380	90

Appendix 6 Supplementary information for Chapter 6. Evaluation of cross-grazing deer with sheep or cattle, as a means to control gastrointestinal and pulmonary nematodes in deer

6.1. Data- Liveweight and Anthelmintic Treatment

Inv= Invermay, Msy= Massey University Palmerston North, anim= Identification number of the deer, f= female, m= male, treat= treatment group, DD= deer own its own, DC= deer cross-grazing with cattle, DS= deer cross-grazing with sheep, SP= suppressively treated deer own its own, lw+n°= liveweight at fortnightly week and tr+n°= treatment at fortnightly week, if tr+n°= 0 not treated and tr+n°=1 treated.

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Inv	303	f	DC	44.7	1	49.4	0	50	1	54.2	0	56.2	0	58.2	1	59	0	61.4	0	61	1
2012	Inv	307	f	DC	43.6	1	48.5	0	51	0	55.4	0	56.4	1	58.6	1	61	0	63.4	0	62.8	1
2012	Inv	308	f	DC	43.6	1	47.9	0	50.5	0	53.2	1	53.6	1	57.6	0	58.8	0	59.8	1	58.8	1
2012	Inv	312	m	DC	44.3	1	47.9	0	50.5	0	56	0	55.6	1	59.8	0	60.8	0	63	0	62	1
2012	Inv	339	f	DC	42.5	1	47.1	0	48	0	52.8	0	53.2	1	55.8	0	55.4	1	57	0	56	1
2012	Inv	341	m	DC	48.8	1	52.2	0	55	0	59	1	59.6	1	62	0	62.8	0	64.6	0	63.8	1
2012	Inv	343	f	DC	45.3	1	49.2	0	51.5	0	54.4	1	55.2	1	56.4	1	58.8	0	59.4	1	59.6	0
2012	Inv	345	m	DC	.	1	53.6	0	55.5	0	61.2	0	60.4	1	63.6	0	63.6	1	65.2	0	65.2	1
2012	Inv	369	m	DC	47	1	52.5	0	55	0	59.4	0	58.4	1	62.2	0	62.2	1	64.2	0	65.2	0
2012	Inv	420	m	DC	43.6	1	47.2	0	50.5	0	53.8	1	54.8	1	57.8	0	59.2	0	60.2	1	59.6	1
2012	Inv	421	m	DC	46.5	1	51	0	52.5	0	57	0	57.6	1	60.8	0	60.4	1	61.2	1	60.4	1
2012	Inv	426	f	DC	50.2	1	56.3	0	59.5	0	64.6	0	65	1	67.2	1	68.6	0	70.8	0	69	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Inv	441	f	DC	48.6	1	54.8	0	56.5	0	60.6	0	61.2	1	63.2	1	63.4	0	65	0	64	1
2012	Inv	447	m	DC	38.4	1	41.5	0	45	0	49.5	0	49.1	1	53.4	0	55	0	56.4	0	52.4	1
2012	Inv	454	f	DC	40.3	1	41.5	0	44	0	45.7	0	46	1	46.7	1	47.7	0	49.4	0	49	1
2012	Inv	464		DC	39.7	1	41.7	0	45.5	0	49.3	0	50.8	0	55.8	0	56.8	0	58	1	58.2	0
2012	Inv	465	m	DC	51	1	56.3	0	58	0	63.2	0	63.6	1	68.8	0	69.4	0	71	1	70.8	1
2012	Inv	467	m	DC	54.4	1	58.4	0	62	0	68	0	68	1	73.4	0	74.2	0	74.4	1	74.6	0
2012	Inv	478	m	DC	46.7	1	51.7	0	51.5	1	57.4	0	59	0	61.4	0	62.6	0	65.2	0	62.8	1
2012	Inv	324	f	DD	46.8	1	47.3	0	53.6	0	54.6	1	56.6	0	58.8	0	57.6	1	61.2	0	59.4	1
2012	Inv	325	f	DD	50.1	1	53.7	0	60	0	61.4	1	63.4	0	65.4	1	66.2	0	67.8	0	66.6	1
2012	Inv	327	f	DD	46.3	1	46.2	0	52.6	0	56.2	1	57	1	60.8	0	60.2	1	63.2	0	62.8	1
2012	Inv	331	m	DD	44	1	46.5	0	53.4	0	55.2	1	58	0	61.6	0	61	1	65.2	0	64.6	1
2012	Inv	333	f	DD	46.8	1	47.6	0	51.6	0	51.6	1	54	0	57	0	56	1	59.8	0	60.4	0
2012	Inv	342	m	DD	50.4	1	53.4	0	56.2	0	58.8	1	60.4	0	62	1	62.2	0	65.6	0	64.6	1
2012	Inv	349	m	DD	49.7	1	52.7	0	57.4	0	59.2	1	62.8	0	65.2	1	66.6	0	69.6	0	70	0
2012	Inv	352	f	DD	41.9	1	43.7	0	46.8	0	48.7	1	51.2	0	52.4	1	54	0	56	0	54.6	1
2012	Inv	359	f	DD	40.8	1	42.5	0	47.2	0	49	1	50	0	53.2	0	52	1	54.6	0	55.4	0
2012	Inv	364	f	DD	52.4	1	55.2	0	59.4	0	62.2	1	62.6	1	67	0	65.2	1	69	0	69.2	0
2012	Inv	424	m	DD	42.2	1	44.6	0	49.3	0	50.6	1	52.6	0	55.4	0	55.4	1	57	0	56.8	1
2012	Inv	425	m	DD	45.7	1	47.4	0	51.4	0	52.4	1	54.2	0	56.4	0	56.4	1	58.8	0	58.8	1
2012	Inv	427	m	DD	41.7	1	44.6	0	50.4	0	50.8	1	52.4	0	55.6	0	55.6	1	60.6	0	59.8	1
2012	Inv	430	m	DD	49.2	1	52.2	0	57.6	0	60.2	1	63.8	0	66.6	0	66.8	0	69	0	66.6	1
2012	Inv	448	m	DD	48.3	1	48.4	0	53.6	0	53.4	1	54.6	0	56.8	0	57.2	0	57.2	1	57.4	0
2012	Inv	449	m	DD	46.8	1	48	0	55	0	55.6	1	59.6	0	62.8	0	63	0	63.2	1	65.2	0
2012	Inv	468	f	DD	44.2	1	46.3	0	53.6	0	56.8	1	59.8	0	62.4	0	61.6	1	65.4	0	64.6	1
2012	Inv	471	m	DD	39.6	1	42.1	0	45.5	0	45.2	1	47.8	0	50.8	0	51.6	0	52	1	52	1
2012	Inv	476	f	DD	40.9	1	40.9	0	45.3	0	44.9	1	48.3	0	51	0	49.6	1	53.4	0	53.2	1
2012	Inv	305	m	DS	46.7	1	48.8	0	51.4	0	50.8	1	55	0	57.6	0	56	1	58	0	57.4	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Inv	309	f	DS	44.1	1	47	0	52.8	0	50.8	1	53	0	57.8	0	57.4	1	56.6	1	58.6	0
2012	Inv	311	f	DS	.	1	52.9	0	55.6	0	55.6	1	57.4	0	61.4	0	61	1	62.4	1	62.8	0
2012	Inv	314	f	DS	41.9	1	46.9	0	49.3	0	49.9	1	51	0	54.4	0	54.2	1	54	1	55	0
2012	Inv	319	m	DS	46.9	1	49.2	0	50.8	0	51	1	52.4	0	55.6	0	54	1	55.6	0	55.6	1
2012	Inv	329	f	DS	48.3	1	51	0	53.6	0	54.4	1	54.8	1	56.6	1	56	1	57.2	1	57.8	0
2012	Inv	337	f	DS	42.8	1	46.9	0	48.8	0	48.5	1	50.8	0	52.8	0	53	0	53.2	1	54.2	0
2012	Inv	344	m	DS	52.8	1	56.6	0	59	0	55.2	1	57.2	0	61.2	0	59.2	1	62.6	0	62.8	0
2012	Inv	346	m	DS	.	1	56.6	0	59.4	0	57	1	59	0	62.2	0	64.6	0	65.2	1	63.8	1
2012	Inv	354	f	DS	39.8	1	42	0	43.1	0	39.6	1	41	0	44.3	0	44	1	45.1	0	46	0
2012	Inv	360	m	DS	44.1	1	49.4	0	51	0	50.6	1	52.8	0	57.2	0	56.4	1	58.8	0	60.4	0
2012	Inv	361	m	DS	43.5	1	48.4	0	49.7	0	46.5	1	46.7	1	50.6	0	51.4	0	51.6	1	50.8	1
2012	Inv	370	f	DS	46.1	1	49.3	0	52.2	0	45.8	1	48.5	0	51.2	0	51.6	0	52	1	53.6	0
2012	Inv	371	f	DS	44.4	1	46.6	0	49.1	0	50.4	1	51.8	0	54.6	0	54.8	0	55.4	1	56	0
2012	Inv	434	m	DS	45.1	1	50.7	0	51.6	1	51.8	1	54.4	0	55.8	1	54.6	1	56.6	0	55.6	1
2012	Inv	450	m	DS	41.6	1	47.7	0	46.7	1	50	1	51.2	0	52.4	1	51.6	1	53	0	53.6	0
2012	Inv	462	m	DS	48.5	1	53.5	0	55	0	43.2	1	.	0	.	.	.	.	.	0	.	.
2012	Inv	463	m	DS	45.1	1	51	0	48.9	1	49.6	1	52	0	52	1	52.8	0	53.6	1	54	0
2012	Inv	472	f	DS	43.7	1	43.8	0	46.9	0	45.9	1	48.3	0	50	1	50.4	0	51.4	1	53	0
2012	Inv	321	m	SP	49.9	1	54.7	1	57	1	62.6	1	64.4	1	68.8	1	68.8	1	72.6	1	70.8	1
2012	Inv	322	m	SP	43.4	1	49.9	1	51.5	1	55.4	1	60	1	64.2	1	66.2	1	66.8	1	68	1
2012	Inv	328	m	SP	48	1	55.5	1	57	1	62.2	1	62.2	1	66.8	1	66.8	1	68.8	1	68.8	1
2012	Inv	330	m	SP	49.9	1	56.6	1	58.5	1	63.2	1	64	1	68.8	1	67.4	1	68.4	1	69	1
2012	Inv	336	m	SP	47.3	1	53	1	52.5	1	57.8	1	59	1	62	1	62.4	1	64.2	1	64.6	1
2012	Inv	356	f	SP	44.3	1	49.8	1	51	1	55.2	1	58.6	1	61.4	1	61.8	1	62.8	1	63.4	1
2012	Inv	357	f	SP	44	1	47.2	1	49.5	1	55.8	1	56	1	61.4	1	61.4	1	62.4	1	63.6	1
2012	Inv	363	f	SP	43.9	1	51.4	1	51	1	55.6	1	56.8	1	60	1	58.8	1	60.6	1	60.2	1
2012	Inv	366	m	SP	45.5	1	52.7	1	54.5	1	57	1	58.6	1	63.2	1	62.8	1	63.8	1	65.2	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Inv	367	f	SP	46.8	1	52.2	1	53	1	57.8	1	60	1	62.8	1	63.6	1	65	1	65.2	1
2012	Inv	418	m	SP	45.8	1	50.7	1	52	1	58	1	60.6	1	65.4	1	66.2	1	67.4	1	67.4	1
2012	Inv	443	f	SP	41.8	1	47.3	1	49.5	1	53.8	1	55.4	1	58.6	1	59	1	60.6	1	60	1
2012	Inv	453	m	SP	52.5	1	58.2	1	60	1	65	1	65.6	1	68	1	69.4	1	70.2	1	68.8	1
2012	Inv	459	f	SP	.	1	45.9	1	48.5	1	52.8	1	54.8	1	59	1	59.2	1	61.4	1	60.6	1
2012	Inv	460	f	SP	42.4	1	48.4	1	50.5	1	56	1	56.6	1	61	1	61.2	1	63.8	1	63.8	1
2012	Inv	466	f	SP	46.2	1	49.6	1	51	1	56.4	1	56.6	1	59.8	1	60.4	1	62.2	1	61.6	1
2012	Inv	469	f	SP	41.7	1	46.3	1	45.5	1	49.3	1	52.2	1	56.8	1	57.4	1	59.2	1	60.2	1
2012	Inv	473	m	SP	44.3	1	47.8	1	49.5	1	55.6	1	56	1	60	1	59	1	59.8	1	60.4	1
2012	Inv	481	m	SP	57.9	1	65.2	1	67	1	72.2	1	74	1	78.2	1	78.8	1	82.8	1	83.2	1
2012	Msy	9	m	DC	37.5	1	42.5	0	46	0	50.2	0	52.8	0	56	0	56.5	0	60	0	59	1
2012	Msy	14	m	DC	61.5	1	65	0	67	1	70.6	0	73.4	0	75.5	0	77	0	78.5	0	77.5	1
2012	Msy	23	f	DC	53.5	1	57.5	0	58	1	61.5	0	62.6	1	66.5	0	64.5	1	68.5	0	67	1
2012	Msy	25	f	DC	55	1	62	0	63	1	67.4	0	70.4	0	73	0	72	1	77	0	75.5	1
2012	Msy	29	m	DC	52.5	1	55	0	59	0	62.4	0	63	1	67	0	66	1	68	0	68.5	1
2012	Msy	30	m	DC	43.5	1	48.5	0	51.5	0	55.2	0	58.4	0	61.5	0	61	1	64.5	0	64.5	1
2012	Msy	31	f	DC	40	1	44.5	0	47	0	50.2	0	52.8	0	55.5	0	56	0	57.5	0	57	1
2012	Msy	32	f	DC	41.5	1	46	0	49.5	0	53.8	0	57	0	58.5	0	60	0	62	0	60	1
2012	Msy	39	m	DC	49.5	1	55.5	0	57	1	60.8	0	62.2	1	66	0	68	0	69	0	64	1
2012	Msy	47	f	DC	55	1	60	0	60.5	1	66.2	0	68.8	0	72	0	71	1	74	0	74	1
2012	Msy	51	m	DC	58.5	1	67.5	0	67.5	1	62.8	1	76.2	0	69	1	78.5	0	75.5	1	79	1
2012	Msy	54	m	DC	65.5	1	72	0	73.5	1	77.4	0	80	0	83.5	0	81.5	1	85	0	83.5	1
2012	Msy	56	f	DC	59.5	1	64	0	66.5	0	70.6	0	73	0	76	1	75	1	79	0	78.5	1
2012	Msy	57	m	DC	54	1	60	0	62	0	65.4	0	68.4	0	71.5	0	70.5	1	74.5	1	74	1
2012	Msy	63	m	DC	59	1	65	0	67.5	0	70.8	0	72.8	1	75.5	0	75	1	75.5	0	78	1
2012	Msy	65	f	DC	45.5	1	51.5	0	54.5	0	56.8	0	58.2	1	60.5	0	60	1	65.5	0	63.5	1
2012	Msy	66	m	DC	41.5	1	47.5	0	50	0	53.8	0	54.8	1	58	0	57.5	1	61	0	61.5	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Msy	74	f	DC	51	1	56	0	57.5	1	61.6	0	63.2	1	67	0	67	1	70.5	0	70	1
2012	Msy	75	f	DC	49.5	1	55	0	57	0	60	0	62.8	0	64.5	1	64	1	68	0	67.5	1
2012	Msy	84	m	DC	55.5	1	61.5	0	64.5	0	68	0	69.6	1	72	0	73	0	75	0	73.5	1
2012	Msy	5	m	DD	41.5	1	46	0	48.5	0	51	1	54.4	0	54.5	1	56.5	0	60	0	59	1
2012	Msy	10	f	DD	49	1	53	0	55	0	53	1	56.8	0	57.5	1	59	0	58	1	61	1
2012	Msy	16	f	DD	39.5	1	42	0	43.5	0	45	0	45.4	1	46.5	0	46.5	1	50	0	48	1
2012	Msy	21	f	DD	60.5	1	65.5	0	64.5	1	67	0	68.2	1	69	1	70	0	69	1	70	1
2012	Msy	22	m	DD	64	1	68.5	0	71	0	69	1	73	0	69	1	72	0	76.5	0	73.5	1
2012	Msy	33	f	DD	64.5	1	69.5	0	69.5	1	71	1	73	1	74	1	76	0	78	0	79.5	1
2012	Msy	34	m	DD	56.5	1	62	0	62.5	1	66	0	69	0	66	1	67.5	0	69.5	0	67.5	1
2012	Msy	44	m	DD	53.5	1	57	0	58.5	1	61	0	62.4	1	64	0	64	1	68	0	68	1
2012	Msy	52	f	DD	53.5	1	58	0	58	1	60	0	62	0	61	1	62.5	0	67	0	65.5	1
2012	Msy	60	f	DD	54	1	58	0	57.5	1	59	1	62	0	60	1	62	0	64.5	0	63.5	1
2012	Msy	67	f	DD	44	1	47	0	47	1	51	0	52.6	1	55	0	54.5	1	57.5	0	57	1
2012	Msy	68	m	DD	43.5	1	50	0	52	0	53	1	56.4	0	55	1	58.5	0	63	0	62	1
2012	Msy	69	m	DD	63.5	1	66	0	67	1	68	1	72.4	0	69.5	1	74	0	78.5	0	77	1
2012	Msy	70	m	DD	54	1	56.5	0	57.5	1	60	0	62.4	0	61	1	64	0	69	0	66	1
2012	Msy	73	f	DD	56	1	60	0	61	1	64	0	65.8	1	67.5	0	68	0	70	0	71.5	1
2012	Msy	76	f	DD	55	1	60	0	59	1	60	1	62.8	0	61	1	62	0	66	0	64	1
2012	Msy	77	m	DD	54	1	59	0	58.5	1	63	0	63.8	1	66	0	65.5	1	71	0	70.5	1
2012	Msy	78	m	DD	57.5	1	54.5	0	55.5	1	59	0	59.8	1	61	1	63	0	64.5	0	63.5	1
2012	Msy	80	m	DD	49.5	1	51.5	0	51.5	1	56	0	55.2	1	54.5	1	57.5	0	60	0	59.5	1
2012	Msy	82	f	DD	49.5	1	52	0	53.5	1	56	0	56.8	1	59.5	0	59	1	62.5	0	60	1
2012	Msy	3	f	DS	54.5	1	58.5	0	61.5	0	56	1	61.8	0	62	1	62	1	68	1	68.5	1
2012	Msy	11	f	DS	49.5	1	51	0	51.5	1	52	1	54.8	0	57	0	57	1	59.5	0	58	1
2012	Msy	12	m	DS	53.5	1	54	0	57.5	0	54	1	68.2	0	60	1	61	0	66	0	64	1
2012	Msy	13	m	DS	64	1	67	0	69	1	67	1	70	0	70	1	71	0	75.5	0	73	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Msy	15	f	DS	40.5	1	44.5	0	45.5	1	44	1	47.5	0	47	1	47.5	0	50.5	0	50	1
2012	Msy	20	f	DS	59.5	1	63	0	61.5	1	64	0	66.6	0	65.5	1	66.5	0	71	0	69	1
2012	Msy	24	m	DS	42.5	1	48	0	51	0	48	1	51.6	0	53.5	0	53.5	1	56.5	0	57.5	1
2012	Msy	26	m	DS	56.5	1	59.5	0	60	1	60	1	63.2	0	63	1	63.5	0	67	0	65.5	1
2012	Msy	28	f	DS	55	1	58	0	60	0	59	1	61.6	0	63.5	0	63	1	66	0	66	1
2012	Msy	37	f	DS	56	1	59	0	61.5	0	45	1	62.2	0	65.5	0	65	1	68	0	68.5	1
2012	Msy	38	m	DS	58.5	1	62	0	63	1	64	1	65.2	1	68.5	0	68	1	70.5	0	72.5	1
2012	Msy	40	m	DS	49	1	52.5	0	55	0	53.8	1	54	1	57	0	58	0	60.5	0	54.5	1
2012	Msy	42	f	DS	51	1	53.5	0	55.5	0	54	1	55.4	1	57.5	0	56	1	60	0	60	1
2012	Msy	45	m	DS	60.5	1	63.5	0	61.5	1	61	1	64.8	0	65	1	65.5	0	70	0	72	1
2012	Msy	48	m	DS	57	1	60.5	0	61	1	62.6	1	65.2	0	66.5	1	67	0	67.5	0	64.5	1
2012	Msy	50	m	DS	54.5	1	59	0	60	1	58.8	1	60.8	0	61.5	1	60.5	1	65	0	64	1
2012	Msy	55	f	DS	42	1	45.5	0	45	1	45.2	1	49.7	0	50	1	50.5	0	55.5	0	52.5	1
2012	Msy	59	f	DS	44.5	1	49	0	51.5	0	48	1	49.5	1	49.5	1	49	1	55	0	56.5	1
2012	Msy	71	m	DS	41.5	1	45.5	0	47	0	46	1	48.8	0	51	0	50.5	1	54	0	52.5	1
2012	Msy	81	m	DS	43	1	47.5	0	48.5	1	49.6	1	49.7	1	51.5	0	51	1	55.5	0	54.5	1
2012	Msy	1	f	SP	40	1	43.5	1	45	1	48	1	50.2	1	51.5	1	55	1	54	1	57	1
2012	Msy	4	f	SP	59.5	1	63	1	64	1	67.2	1	71	1	71	1	70.5	1	71.5	1	74	1
2012	Msy	6	m	SP	46.5	1	51	1	54	1	58	1	58.8	1	61	1	63	1	63.5	1	64.5	1
2012	Msy	7	m	SP	59	1	62.5	1	65.5	1	69	1	73	1	74.5	1	76	1	75	1	79	1
2012	Msy	18	m	SP	43	1	46	1	48	1	51.6	1	54.2	1	56	1	57	1	58.5	1	62	1
2012	Msy	19	m	SP	53.5	1	56.5	1	57.5	1	63	1	65.8	1	66.5	1	68.5	1	69.5	1	75	1
2012	Msy	27	f	SP	48.5	1	56	1	59.5	1	62.4	1	65.4	1	66.5	1	65.5	1	64	1	65	1
2012	Msy	35	f	SP	42.5	1	44.5	1	46.5	1	50.8	1	51.4	1	53	1	55.5	1	55	1	56.5	1
2012	Msy	36	m	SP	57.5	1	62.5	1	64.5	1	66.8	1	69	1	72	1	71.5	1	73	1	77.5	1
2012	Msy	41	f	SP	54.5	1	60	1	59.5	1	63.4	1	65.4	1	67	1	68.5	1	67.5	1	71.5	1
2012	Msy	43	f	SP	54	1	58.5	1	60.5	1	64	1	64	1	69	1	69.5	1	71	1	75	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2012	Msy	46	m	SP	50.5	1	55	1	59	1	63.4	1	64.8	1	67.5	1	67.5	1	68	1	72	1
2012	Msy	49	f	SP	48	1	46	1	.	0	.	.	.	.	.	.	.	.	.	.	.	.
2012	Msy	53	m	SP	57	1	59.5	1	60.5	1	62.4	1	64.2	1	66	1	63	1	65	1	70.5	1
2012	Msy	61	m	SP	65	1	67	1	72.5	1	77	1	78.8	1	79.5	1	78.5	1	80.5	1	85	1
2012	Msy	62	f	SP	62	1	66	1	69	1	72	1	74.6	1	76	1	73.5	1	72.5	1	76	1
2012	Msy	64	m	SP	48	1	53	1	56	1	59.2	1	61.6	1	63.5	1	64.5	1	64	1	67	1
2012	Msy	72	m	SP	54	1	60	1	62.5	1	66	1	68.2	1	68.5	1	72	1	71.5	1	75	1
2012	Msy	83	f	SP	46	1	49.5	1	51	1	53.2	1	54.9	1	57	1	55.5	1	59.5	1	62.5	1
2012	Msy	85	f	SP	53.5	1	58.5	1	60.5	.	63	1	67.2	1	70	1	69.5	1	70	1	72.5	1
2013	Inv	239	m	DC	60	1	64.5	0	62.5	0	66.5	0	67	1	69.5	0	71.5	0	73.5	0	73.5	1
2013	Inv	251	m	DC	54	1	53.5	0	53.5	0	53	1	55	1	56.5	0	56.5	0	56	1	57.5	0
2013	Inv	262	m	DC	54	1	57.5	0	58.5	0	57.5	1	59.5	1	61	0	61.5	0	62.5	1	61	1
2013	Inv	270	m	DC	42.5	1	46.5	0	46	0	49	0	51	1	54	0	54.5	0	57	0	55.5	1
2013	Inv	281	m	DC	50.5	1	53.5	0	51.5	0	55.5	0	50	1	55	0	54	0	55.5	0	57.5	1
2013	Inv	109	m	DC	58.5	1	62.5	0	61.5	0	64	0	63.5	1	66	0	66	0	67.5	0	67.5	1
2013	Inv	118	m	DC	48	1	53	0	52	0	55	0	52	1	57	0	58	0	57	1	56	1
2013	Inv	125	m	DC	47.5	1	51.5	0	51	0	54	0	52.5	1	53	1	.	.	.	.	.	.
2013	Inv	132	m	DC	45.5	1	48	0	47	0	49	0	49	1	50	1	48	1	49.5	0	52	0
2013	Inv	145	m	DC	52	1	54.5	0	53	0	55.5	0	54	1	59	0	56	1	58	0	59	0
2013	Inv	149	m	DC	52.5	1	54.5	0	55	0	56.5	1	55.5	1	58	0	55.5	1	58	0	57.5	1
2013	Inv	170	m	DC	57.5	1	60	0	60	0	62.5	0	63	1	64.5	0	64.5	1	65	1	63.5	1
2013	Inv	188	m	DC	60	1	62.5	0	60.5	0	64	0	62.5	1	65.5	0	66	0	67	1	70	0
2013	Inv	202	m	DC	61.5	1	65	0	63	0	65	1	61	1	65	0	65	0	64	1	63.5	1
2013	Inv	217	m	DC	56	1	59	0	56.5	0	59	0	56.5	1	60	0	58	1	59.5	0	59	1
2013	Inv	223	m	DC	55.5	1	60	0	61.5	0	62.5	1	63	1	66	0	67	0	67.5	1	67.5	1
2013	Inv	270	m	DC	53.5	1	58	0	55.5	0	58	0	58.5	1	59	1	59	0	61.5	0	61	1
2013	Inv	277	m	DC	48.5	1	50.5	0	50.5	0	53.5	0	53.5	1	56.5	0	58.5	0	58.5	1	59	0

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2013	Inv	282	m	DC	52	1	54	0	52	0	55	0	53	1	56.5	0	55.5	0	56.5	0	57.5	0
2013	Inv	212	m	DD	59	1	66	0	64	0	66.5	1	65.5	1	67	1	65.5	0	67	0	65	1
2013	Inv	230	m	DD	57	1	60.5	0	59	0	59.5	1	58	1	58	1	58	0	59	0	57	1
2013	Inv	242	m	DD	51	1	55.5	0	55.5	0	56.5	1	57	0	61.5	0	58.5	1	61.5	0	60	1
2013	Inv	244	m	DD	49.5	1	54.5	0	53.5	0	56	0	55.5	1	58	0	55.5	1	59.5	0	59	1
2013	Inv	245	m	DD	50	1	55.5	0	55	0	56.5	1	56.5	1	58.5	0	56.5	1	60	0	59.5	1
2013	Inv	246	m	DD	53.5	1	55.5	0	53.5	0	53.5	1	54	1	56	0	54.5	0	54.5	1	55.5	1
2013	Inv	58	m	DD	51	1	58	0	55.5	0	57	1	58.5	0	58	1	57	0	59	0	58	1
2013	Inv	267	m	DD	40.5	1	46.5	0	45.5	0	45	1	45.5	0	48.5	0	46.5	1	49	0	49	1
2013	Inv	105	m	DD	54.5	1	59.5	0	58	0	58	1	58.5	0	59.5	1	56	1	60	0	60.5	0
2013	Inv	107	m	DD	58.5	1	65	0	64.5	0	64	1	63.5	1	64.5	1	63.5	0	65.5	0	61	1
2013	Inv	111	m	DD	53.5	1	60	0	58.5	0	59.5	1	60	0	63.5	0	61.5	1	63	0	63.5	0
2013	Inv	122	m	DD	42	1	47	0	48	0	48	1	48	0	48.5	1	50.5	0	47.5	1	48	0
2013	Inv	131	m	DD	52.5	1	57	0	57.5	0	57	1	58	0	58.5	1	55.5	1	58.5	0	58	1
2013	Inv	136	m	DD	49.5	1	53	0	52.5	0	53	1	52.5	1	54.5	0	52	1	53	0	52.5	1
2013	Inv	152	m	DD	56	1	60.5	0	59	0	61	1	60.5	1	63	0	61	1	62	1	60.5	1
2013	Inv	234	m	DD	48	1	53.5	0	53	0	52	1	50.5	1	54	0	52	1	53.5	0	51.5	1
2013	Inv	239	m	DD	53.5	1	61.5	0	58	0	60.5	0	58.5	1	60	1	58.5	0	62	0	61.5	1
2013	Inv	242	m	DD	56.5	1	62	0	64	0	63	1	65	0	67	0	64.5	1	67.5	0	66.5	1
2013	Inv	258	m	DD	43	1	48	0	39	1	.	.	.	.	.	.	.	.	.	.	.	.
2013	Inv	232	m	DS	40.5	1	45.5	0	45.5	0	46.5	1	46	1	47	1	49	0	49.5	1	51	0
2013	Inv	248	m	DS	51	1	57.5	0	57.5	0	60	0	59.5	1	60.5	1	62	0	62.5	1	63	1
2013	Inv	254	m	DS	46.5	1	53.5	0	51.5	0	52.5	1	52.5	0	52.5	1	51	0	53	0	52.5	1
2013	Inv	260	m	DS	51.5	1	55	0	54.5	0	57	0	57	0	56	1	59	0	59	1	59.5	0
2013	Inv	272	m	DS	55	1	60.5	0	59.5	0	62.5	0	65	1	64	1	67	0	69.5	0	69.5	1
2013	Inv	93	m	DS	39	1	44.5	0	44	0	45	1	45.5	0	45.5	1	46	0	45.5	1	46	0
2013	Inv	115	m	DS	58	1	62	0	60	0	62	1	63	0	63.5	1	64.5	0	65.5	1	64	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2013	Inv	127	m	DS	45	1	49.5	0	47.5	0	48	1	48	1	50.5	0	52	0	53.5	0	55	0
2013	Inv	138	m	DS	50	1	54	0	54.5	0	55	1	57	0	57.5	1	61	0	63	0	62.5	1
2013	Inv	144	m	DS	42	1	48	0	47	0	48	1	48	1	48	1	49	0	50.5	0	50.5	1
2013	Inv	148	m	DS	54.5	1	60.5	0	59	0	62	0	63	1	55.5	1	57.5	0	60	0	60	1
2013	Inv	157	m	DS	60	1	66.5	0	63.5	0	65.5	1	61.5	1	63.5	0	66.5	0	67	1	68.5	0
2013	Inv	158	m	DS	58.5	1	61.5	0	60.5	0	63.5	0	63.5	0	64	1	65.5	0	66.5	1	65.5	1
2013	Inv	191	m	DS	56	1	61.5	0	60.5	0	60.5	1	59.5	1	59.5	1	57	1	58	0	58	1
2013	Inv	206	m	DS	58.5	1	66.5	0	63.5	0	65	1	64.5	1	62.5	1	64.5	0	65	1	63.5	1
2013	Inv	243	m	DS	50.5	1	55.5	0	54.5	0	56.5	1	58	0	60	0	62	0	61	1	61	1
2013	Inv	259	m	DS	48.5	1	54	0	54	0	56	1	55.5	1	56	1	57	0	58	0	58	1
2013	Inv	280	m	DS	47	1	52	0	50.5	0	53.5	0	53	1	54.5	0	56	0	56.5	1	57.5	0
2013	Inv	319	m	DS	54.5	1	59.5	0	59	0	61.5	0	58	1	60.5	0	60	0	61	1	61	1
2013	Inv	90	m	SP	48.5	1	54	1	54	1	58	1	54	1	58	1	58	1	59.5	1	60.5	1
2013	Inv	103	m	SP	54	1	58.5	1	57.5	1	60.5	1	60.5	1	61.5	1	63	1	63	1	63.5	1
2013	Inv	116	m	SP	56.5	1	62	1	62.5	1	66.5	1	67	1	67.5	1	66	1	67	1	67.5	1
2013	Inv	129	m	SP	41.5	1	46	1	46	1	49	1	47.5	1	49	1	50	1	49.5	1	49.5	1
2013	Inv	130	m	SP	51	1	55	1	55	1	56.5	1	55.5	1	56.5	1	56	1	56.5	1	57.5	1
2013	Inv	135	m	SP	52	1	54.5	1	53.5	1	56.5	1	56	1	56.5	1	56.5	1	57.5	1	57.5	1
2013	Inv	162	m	SP	51.5	1	56	1	53.5	1	57.5	1	56	1	58	1	54.5	1	57.5	1	58.5	1
2013	Inv	165	m	SP	57.5	1	63.5	1	64.5	1	67.5	1	67	1	70	1	68.5	1	68.5	1	69.5	1
2013	Inv	174	m	SP	42.5	1	46	1	45.5	1	48	1	47.5	1	50.5	1	50	1	52	1	52	1
2013	Inv	208	m	SP	59	1	61.5	1	63.5	1	64	1	62	1	65	1	62.5	1	63	1	65.5	1
2013	Inv	216	m	SP	54.5	1	62.5	1	62	1	65	1	63.5	1	65.5	1	61	1	63	1	61.5	1
2013	Inv	247	m	SP	53.5	1	59	1	60	1	63.5	1	64.5	1	66.5	1	65	1	67	1	67	1
2013	Inv	289	m	SP	45.5	1	51.5	1	51.5	1	52.5	1	54.5	1	58	1	56	1	58	1	59.5	1
2013	Inv	294	m	SP	44.5	1	50.5	1	48	1	52.5	1	50	1	53	1	52	1	54	1	54	1
2013	Inv	297	m	SP	47.5	1	55	1	53	1	55.5	1	54.5	1	57	1	55	1	55	1	57	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2013	Inv	301	m	SP	50	1	55	1	55	1	58	1	55.5	1	57	1	55	1	57	1	57	1
2013	Inv	324	m	SP	53.5	1	57.5	1	57.5	1	60.5	1	58	1	59.5	1	56.5	1	60.5	1	60	1
2013	Inv	271	m	SP	54.5	1	61	1	59.5	1	64	1	63.5	1	65	1	65	1	65.5	1	66	1
2013	Inv	275	m	SP	47.5	1	53.5	1	53.5	1	56	1	56.5	1	58	1	55	1	57	1	58.5	1
2013	Msy	205	f	DC	56.5	1	59.5	0	61.5	0	63.5	1	67	0	69	1	73	0	72.5	1	77	0
2013	Msy	212	f	DC	42.5	1	42.5	0	44.5	0	48	0	50	0	52	0	56.5	0	57	1	62.5	0
2013	Msy	218	m	DC	49	1	53	0	54	1	57	1	59	0	60.5	1	67	0	66.5	1	70.5	0
2013	Msy	223	f	DC	59	1	63	0	65	0	67	1	69.5	0	72.5	0	75.5	0	74.5	1	79	0
2013	Msy	227	m	DC	64	1	66.5	0	68	1	67.5	1	72	0	74	1	81	0	77.5	1	83	0
2013	Msy	233	f	DC	61.5	1	64.5	0	66	1	70.5	0	74	0	77	0	82.5	0	82	1	88.5	0
2013	Msy	237	f	DC	55	1	57.5	0	59	1	62.5	1	64.5	0	69	0	74.5	0	77.5	0	82	0
2013	Msy	242	m	DC	48.5	1	52.5	0	54	0	57.5	1	61	0	65	0	70	0	69.5	1	73.5	0
2013	Msy	243	m	DC	60	1	65.5	0	68	0	69	1	72.5	0	76.5	0	81.5	0	83.5	0	89.5	0
2013	Msy	245	m	DC	63	1	66	0	68.5	0	71	1	73.5	0	77	0	80	1	82	0	87	0
2013	Msy	250	m	DC	67	1	70	0	73	0	72	1	74	1	73.5	1	78	0	81	0	88	0
2013	Msy	252	f	DC	55	1	56	0	59.5	0	62.5	1	68	0	71	0	73.5	1	75.5	0	79	0
2013	Msy	253	m	DC	59	1	63.5	0	65.5	0	70.5	0	76	0	76.5	1	80	0	81.5	1	87	0
2013	Msy	255	f	DC	46.5	1	50.5	0	51	1	54	1	57.5	0	59.5	1	63	0	61.5	1	66.5	0
2013	Msy	256	f	DC	47	1	50.5	0	53.5	0	55.5	1	59	0	62.5	0	65.5	0	68	0	70.5	0
2013	Msy	260	m	DC	59	1	61.5	0	63	1	66.5	1	73	0	75	1	76.5	1	84.5	0	90.5	0
2013	Msy	268	f	DC	61	1	66	0	66.5	1	68.5	1	69	1	73.5	0	77	0	78.5	1	83.5	0
2013	Msy	269	m	DC	53.5	1	57	0	61.5	0	65.5	1	70	0	74.5	0	79	0	79.5	1	84	0
2013	Msy	270	m	DC	58.5	1	63.5	0	63	1	66.5	1	69.5	0	74	0	78	0	79.5	1	86.5	0
2013	Msy	278	m	DC	59.5	1	55.5	0	56	1	61.5	0	66	0	70	0	75	0	75.5	1	81.5	0
2013	Msy	201	m	DD	58.5	1	61.5	0	62.5	1	63.5	1	64.5	1	67.5	0	71.5	0	71.5	1	79.5	0
2013	Msy	210	m	DD	62	1	61.5	0	63.5	0	67.5	1	72.5	0	76	0	82.5	0	82.5	1	87.5	0
2013	Msy	215	f	DD	62	1	62.5	0	62.5	1	66	1	67	1	71.5	0	76.5	0	75.5	1	79	0

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2013	Msy	216	f	DD	48	1	53.5	0	54.5	1	56	1	56	1	60	0	62.5	0	65	0	69.5	0
2013	Msy	220	f	DD	55	1	57	0	56.5	1	61.5	0	63	1	68	0	73	0	72.5	1	77.5	0
2013	Msy	221	f	DD	44	1	47	0	48.5	0	52	0	53	1	58.5	0	63	0	64	1	69	0
2013	Msy	228	m	DD	57	1	60.5	0	61.5	1	65	1	65.5	1	72.5	0	75.5	0	75	1	84.5	0
2013	Msy	229	m	DD	46	1	50.5	0	50.5	1	54.5	0	55	1	59	0	66	0	65	1	73	0
2013	Msy	231	m	DD	51.5	1	52	0	55	0	59	0	59	1	65	0	67.5	1	70	0	76.5	0
2013	Msy	236	m	DD	60.5	1	60.5	0	61	1	65	1	67	0	67	1	72	0	73	1	77.5	0
2013	Msy	244	m	DD	58.5	1	60	0	62	0	64	1	65	1	68	0	71	0	72.5	1	78.5	0
2013	Msy	249	m	DD	61	1	64	0	63	1	65.5	1	66.5	1	68.5	1	74.5	0	76.5	0	79	1
2013	Msy	251	f	DD	58	1	61.5	0	61	1	64.5	1	64	1	67.5	0	69.5	1	75	0	75	1
2013	Msy	254	m	DD	58.5	1	60.5	0	62	1	64.5	1	64	1	68	0	71.5	0	73.5	0	74	1
2013	Msy	257	f	DD	48	1	51	0	50.5	1	55.5	0	56.5	1	61.5	0	65	0	66	1	70	0
2013	Msy	258	m	DD	64	1	68.5	0	70.5	0	72.5	1	74.5	1	82	0	85.5	0	86	1	93	0
2013	Msy	264	m	DD	66	1	67.5	0	69.5	0	71	1	72	1	77	0	80	1	82.5	0	88.5	0
2013	Msy	271	m	DD	34	1	36.5	0	34	1	35	1	37.5	0	40.5	0	44.5	0	43.5	1	49	0
2013	Msy	281	f	DD	52	1	50.5	0	52	0	57	0	59	0	62	0	64.5	0	65	1	70	0
2013	Msy	202	f	DS	51	1	53.5	0	55.5	0	59.5	0	59	1	63	0	66	0	69	0	72	0
2013	Msy	203	m	DS	50	1	57.5	0	58	1	60.5	1	63.5	0	68	0	71.5	0	73	1	77	0
2013	Msy	206	m	DS	55	1	68.5	0	67	1	70	1	70	1	73	0	76	0	76	1	77.5	1
2013	Msy	219	m	DS	52	1	54	0	54.5	1	59.5	0	59	1	62	0	65.5	0	70	0	70.5	1
2013	Msy	224	m	DS	64.5	1	69.5	0	68.5	1	73	1	74	1	79	0	84	0	85	1	86.5	1
2013	Msy	225	m	DS	71.5	1	77	0	73.5	1	81	0	80	1	87	0	90.5	0	92.5	1	90.5	1
2013	Msy	226	m	DS	54.5	1	58.5	0	59.5	1	63.5	0	64.5	1	69	0	71	1	76.5	0	77	1
2013	Msy	230	m	DS	65	1	70	0	70	1	74.5	1	74	1	78.5	0	84	0	86	0	89	1
2013	Msy	235	m	DS	45.5	1	47	0	47.5	1	51	0	53.5	0	54.5	1	58	0	61	0	65	0
2013	Msy	238	m	DS	61.5	1	64	0	63	1	68	0	66.5	1	71	0	75	0	77.5	0	78.5	1
2013	Msy	239	m	DS	58.5	1	61	0	61.5	1	66.5	0	67	1	74	0	78	0	80.5	0	85	0

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2013	Msy	240	f	DS	40	1	44.5	0	44	1	47.5	0	50	0	53.5	0	55.5	1	58	0	63	0
2013	Msy	247	f	DS	57.5	1	64	0	65	1	69	1	71	1	74	0	78	0	76.5	1	78.5	1
2013	Msy	259	f	DS	57	1	58.5	0	58.5	1	63	0	64	1	68	0	70	1	72	0	72.5	1
2013	Msy	265	f	DS	55.5	1	61	0	60.5	1	66	0	65.5	1	69.5	0	74	0	76.5	0	77.5	1
2013	Msy	267	m	DS	58.5	1	64	0	67	0	74	0	78	0	83.5	0	85.5	1	90	0	92	1
2013	Msy	272	f	DS	62.5	1	66.5	0	65.5	1	72	0	72.5	1	77	0	78	1	81.5	0	84.5	0
2013	Msy	273	f	DS	54	1	59	0	59	1	64	0	64.5	1	66	1	69.5	0	72.5	0	73.5	1
2013	Msy	277	m	DS	58	1	62.5	0	61.5	1	68.5	0	70	1	74.5	0	79	0	84	0	84.5	1
2013	Msy	280	m	DS	54.5	1	59.5	0	60	1	64	0	63.5	1	66.5	0	68	1	70	0	74	0
2013	Msy	204	m	SP	60.5	1	64	1	65	1	71	1	73.5	1	79.5	1	83.5	1	84	1	89.5	1
2013	Msy	207	f	SP	57	1	58	1	62	1	69	1	73	1	75.5	1	79.5	1	81.5	1	83.5	1
2013	Msy	208	m	SP	51.5	1	53.5	1	55	1	58.5	1	63	1	66	1	70	1	70.5	1	75	1
2013	Msy	209	m	SP	68	1	70	1	73	1	79	1	79	1	81.5	1	85.5	1	89	1	91	1
2013	Msy	211	m	SP	59	1	60.5	1	61.5	1	69	1	73	1	76.5	1	79	1	82	1	84	1
2013	Msy	213	m	SP	59	1	63	1	65	1	71	1	73.5	1	74	1	79	1	83	1	88	1
2013	Msy	214	m	SP	55	1	68	1	70.5	1	75.5	1	79	1	83	1	88.5	1	92.5	1	98	1
2013	Msy	222	f	SP	59	1	60.5	1	62	1	66	1	67.5	1	71	1	74	1	76.5	1	79	1
2013	Msy	232	m	SP	55	1	59	1	60	1	66.5	1	68.5	1	72	1	77.5	1	74	1	79	1
2013	Msy	246	f	SP	42	1	43.5	1	45	1	50	1	53	1	55	1	59	1	61	1	63.5	1
2013	Msy	248	m	SP	49	1	52	1	54	1	57.5	1	61	1	63	1	67	1	66	1	69.5	1
2013	Msy	261	f	SP	65	1	67	1	69	1	73	1	75	1	77.5	1	79.5	1	82	1	87	1
2013	Msy	262	m	SP	40	1	42.5	1	44.5	1	49.5	1	53	1	58	1	60.5	1	65.5	1	70	1
2013	Msy	263	f	SP	47	1	49	1	50.5	1	54	1	55.5	1	56.5	1	60.5	1	62	1	65	1
2013	Msy	266	f	SP	50	1	52	1	53	1	56	1	57.5	1	61	1	63.5	1	67	1	69	1
2013	Msy	274	m	SP	60	1	64	1	67.5	1	72	1	74	1	81	1	84.5	1	89	1	89	1
2013	Msy	275	m	SP	59	1	62.5	1	65.5	1	70.5	1	71.5	1	73	1	73.5	1	75	1	81.5	1
2013	Msy	279	f	SP	59	1	61.5	1	64	1	70	1	71.5	1	75	1	77	1	77.5	1	79	1

year	place	anim	sex	treat	lw0	tr0	lw1	tr1	lw2	tr2	lw3	tr3	lw4	tr4	lw5	tr5	lw6	tr6	lw7	tr7	lw8	tr8
2013	Msy	282	f	SP	50	1	52.5	1	54.5	1	59	1	62	1	66	1	69.5	1	73.5	1	77.5	1
2013	Msy	283	m	SP	57	1	61.5	1	63.5	1	69	1	69.5	1	72	1	76.5	1	78.5	1	80	1

6.2. Data- Faecal egg count and larval count Liveweight and Anthelmintic Treatment

Inv= AgReseach Invermay, Msy= Massey University Palmerston North, anim= Identification number of the deer, treat= treatment group, DD= deer own its own, DC= deer cross-grazing with cattle, DS= deer cross-grazing with sheep, SP= suppressively treated deer own its own, fec+n°= faecal egg count (eggs per gram) at fortnightly week and flc+n°= faecal larval count (larvae per gram) at fortnightly week.

If in purple then the deer had an anthelmintic treatment that day.

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Inv	303	DC	0	0	0	0	150	0	0	0	0	0	0	0	0	0	25	0	50	0
2012	Inv	307	DC	0	0	0	0	0	0	200	0	0	0	0	0	0	0	0	0	100	2
2012	Inv	308	DC	0	0	.	.	0	0	50	.	0	0	0	0	0	0	0	0	0	0
2012	Inv	312	DC	0	0	0	0	350	1.8	150	34	0	0	0	0	0	0	100	0	825	0
2012	Inv	339	DC	.	.	.	.	150	0	50	0	100	0	0	0	0	0	25	0	125	0
2012	Inv	341	DC	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	25	0
2012	Inv	343	DC	0	0	0	0	0	0	0	1	0	0	0	0	.	.	0	0	0	0
2012	Inv	345	DC	0	.	0	0	50	0	50	0	75	0	0	0	0	0	0	0	0	0
2012	Inv	369	DC	0	0	0	0	100	0	25	0	0	0	0	0	50	1	0	0	0	0
2012	Inv	420	DC	0	0	0	0	.	.	100	101	0	0	0	0	50	0	0	0	0	0
2012	Inv	421	DC	.	.	.	.	.	.	50	.	75	0	0	0	0	0	25	0	0	0
2012	Inv	426	DC	0	.	0	0	50	0	75	28	75	0	0	0	0	0	.	.	50	0
2012	Inv	441	DC	0	0	0	0	.	.	0	0	25	0	0	0	0	0	25	0	25	1
2012	Inv	447	DC	0	0	0	.	100	0.3	50	3	0	19	0	0	0	.	50	0	100	0
2012	Inv	454	DC	.	.	0	0	250	11.1	250	76	0	0	0	0	25	0	50	0	225	0
2012	Inv	464	DC	0	0	0	0	50	0	50	0	0	0	75	1	25	0	50	41	0	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Inv	465	DC	0	0	.	.	100	0	75	.	.	.	0	0	50	0	150	0	25	0
2012	Inv	467	DC	.	.	0	0	250	0	125	0	0	0	0	0	0	0	.	.	25	0
2012	Inv	478	DC	0	0	.	.	50	0	200	0	0	0	25	0	0	0	0	0	75	3
2012	Inv	324	DD	0	0	0	0	.	.	450	89	0	0	50	0	0	9	0	0	50	0
2012	Inv	325	DD	0	0	0	.	0	22.5	25	183	0	0	0	0	0	0	0	1	50	1
2012	Inv	327	DD	0	0	0	0	0	0.1	75	41	0	0	0	0	25	2	0	0	25	0
2012	Inv	331	DD	0	0	0	0	.	.	175	172	25	.	0	0	50	58	25	0	50	0
2012	Inv	333	DD	.	.	50	0	50	18.3	325	374	0	0	0	1	0	43	0	3	50	0
2012	Inv	342	DD	0	0	0	.	.	.	.	.	0	0	0	0	0	0	25	4	25	9
2012	Inv	349	DD	.	.	0	0	.	.	0	71	.	0	.	.	.	.	0	0	0	20
2012	Inv	352	DD	0	0	0	0	.	.	100	.	0	0	0	0	0	0	0	0	0	10
2012	Inv	359	DD	0	0	0	0	150	12.8	50	301	0	0	0	0	25	140	.	.	25	0
2012	Inv	364	DD	0	0	0	0	150	0.6	500	136	0	0	0	0	25	0	25	0	25	0
2012	Inv	424	DD	0	0	0	0	200	.	150	50	0	0	0	0	.	.	0	0	.	.
2012	Inv	425	DD	0	0	0	0	200	0	75	29	0	0	0	0	.	.	25	0	0	.
2012	Inv	427	DD	0	0	.	.	50	0.6	100	.	0	0	50	0	75	81	0	0	0	0
2012	Inv	430	DD	0	0	0	0	.	.	300	34	0	0	0	0	0	0	0	92	75	96
2012	Inv	448	DD	0	0	.	.	150	0.2	25	52	.	.	.	.	.	.	.	.	0	0
2012	Inv	449	DD	0	0	0	.	.	.	.	.	.	.	0	0	.	.	25	72	0	0
2012	Inv	468	DD	.	.	0	0	150	0	50	80	0	0	0	0	.	.	.	.	25	0
2012	Inv	471	DD	.	.	.	.	0	2.4	1050	193	0	0	25	0	200	573	625	1077	0	0
2012	Inv	476	DD	0	0	.	.	150	0.4	.	.	0	0	0	0	0	122	0	0	25	0
2012	Inv	305	DS	0	0	.	.	.	.	50	.	0	0	25	0	0	.	.	.	0	0
2012	Inv	309	DS	.	.	0	0	100	0	0	0	0	0	0	0	0	18	0	0	0	0
2012	Inv	311	DS	0	.	0	0	50	0	0	0	0	0	0	0	0	4	0	0	0	0
2012	Inv	314	DS	0	.	0	0	100	32.5	0	34	.	.	0	0	0	8	25	0	0	0
2012	Inv	319	DS	0	0	.	.	.	.	0	113	.	.	0	0	.	.	.	.	0	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Inv	329	DS	0	0	0	0	50	0	25	1	0	0	0	0	0	0	0	0	0	0
2012	Inv	337	DS	0	0	0	0	350	0	125	0	0	.	25	0	0	43	25	1	0	0
2012	Inv	344	DS	.	.	0	0	100	15	300	159	25	0	0	0	25	1	0	0	25	0
2012	Inv	346	DS	0	0	0	0	.	.	250	39	0	0	0	0	0	.	25	6	0	0
2012	Inv	354	DS	0	0	0	0	500	7.1	.	.	0	0	0	0	0	8	0	0	25	2
2012	Inv	360	DS	0	.	0	0	50	0	25	.	0	.	0	0	0	0	0	0	0	0
2012	Inv	361	DS	0	0	0	0	650	48.7	.	.	0	0	0	0	25	0	100	1	0	0
2012	Inv	370	DS	0	0	0	0	800	0	.	.	0	0	0	0	0	0	175	5	0	0
2012	Inv	371	DS	0	0	.	.	150	0.7	0	.	.	.	0	0	0	2	100	1	50	0
2012	Inv	434	DS	0	0	100	0	300	1.1	0	0	0	0	0	0	0	0	0	0	0	0
2012	Inv	450	DS	0	0	50	.	950	0.4	25	0	.	.	25	0	0	0	.	.	.	.
2012	Inv	462	DS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2012	Inv	463	DS	0	.	0	.	.	.	.	.	0	0	0	0	0	0	0	0	25	0
2012	Inv	472	DS	0	.	50	0	350	.	0	27	0	0	25	0	.	.	50	2	0	0
2012	Inv	321	SP	0	0	.	.	0	0	.	.	.	.	.	.	0	0	.	.	25	0
2012	Inv	322	SP	0	0	.	.	.	.	.	.	.	.	.	.	0	0	.	.	.	.
2012	Inv	328	SP	0	0	0	.	.	.	25	0	.	.	.	.	0	0	0	0	.	.
2012	Inv	330	SP	.	.	.	.	0	0	.	.	.	.	0	0	25	0	0	0	0	0
2012	Inv	336	SP	0	0	.	.	.	.	.	.	0	0	.	.	.	.	.	.	25	0
2012	Inv	356	SP	0	0	0	0	.	.	.	.	.	.	.	.	0	0	0	0	.	.
2012	Inv	357	SP	0	0	.	.	.	.	.	.	.	.	0	0	0	0	.	.	25	0
2012	Inv	363	SP	0	0	.	.	.	.	.	.	.	.	0	0	.	.	.	.	0	0
2012	Inv	366	SP	0	0	0	0	.	.	.	.	.	.	.	.	0	0	.	.	0	0
2012	Inv	367	SP	.	.	.	.	.	.	.	.	.	.	.	.	0	0	25	0	.	.
2012	Inv	418	SP	0	0	.	.	50	0	.	.	0	0	.	.	0	0	.	.	0	0
2012	Inv	443	SP	0	0	.	.	0	0	.	.	0	0	.	.	0	0	.	.	.	.
2012	Inv	453	SP	.	.	0	0	.	.	0	0	.	.	.	.	.	.	.	.	.	.

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Inv	459	SP	0	0	0	0	.	.	.	.	.	.	.	.	0	0	.	.	.	.
2012	Inv	460	SP	0	0	.	.	.	.	.	.	.	.	.	.	0	0	.	.	0	0
2012	Inv	466	SP	0	0	.	.	.	.	.	.	.	.	.	.	0	0	.	.	.	.
2012	Inv	469	SP	0	0	.	.	0	0	.	.	.	.	.	.	0	0	.	.	.	.
2012	Inv	473	SP	0	0	.	.	.	.	0	0	.	.	.	.	0	0	.	.	0	0
2012	Inv	481	SP	0	0	.	.	.	.	.	.	.	.	.	.	0	0	.	.	0	0
2012	Msy	9	DC	.	.	0	.	0	0	50	0	100	0	150	3	0	12	0	0	100	187
2012	Msy	14	DC	.	.	0	.	0	0	0	0	0	0	200	3	350	9	.	.	350	2072
2012	Msy	23	DC	.	.	0	.	0	0	0	0	0	0	.	.	0	0	0	0	50	0
2012	Msy	25	DC	.	.	0	.	.	.	0	0	0	0	.	.	150	0	.	.	0	8
2012	Msy	29	DC	.	.	0	.	0	0	150	18	100	86	0	0	56	0	0	0	50	0
2012	Msy	30	DC	.	.	0	.	0	0	50	46	0	29	200	90	50	11	0	0	0	0
2012	Msy	31	DC	.	.	0	.	0	0	100	9	0	10	50	65	0	43	50	27	200	238
2012	Msy	32	DC	.	.	0	.	50	0	0	14	200	72	150	136	50	99	50	389	250	565
2012	Msy	39	DC	.	.	0	.	.	.	0	0	0	0	0	0	0	0	.	.	1250	118
2012	Msy	47	DC	.	.	0	.	0	0	0	0	0	0	50	0	100	1	0	0	50	0
2012	Msy	51	DC	.	.	0	.	0	0	0	0	50	0	0	0	0	0	0	0	0	0
2012	Msy	54	DC	.	.	0	.	0	0	0	0	0	0	0	8	100	3	0	0	0	0
2012	Msy	56	DC	.	.	0	.	0	0	50	132	250	97	250	465	0	0	0	0	0	0
2012	Msy	57	DC	.	.	0	.	0	.	50	38	50	25	250	5	0	1	.	.	0	0
2012	Msy	63	DC	.	.	0	.	0	0	200	32	150	23	0	0	50	0	50	0	0	0
2012	Msy	65	DC	.	.	0	.	0	.	333	80	150	118	0	0	0	2	0	0	0	52
2012	Msy	66	DC	.	.	0	.	0	0	200	4	100	6	0	0	0	0	0	0	0	0
2012	Msy	74	DC	.	.	0	.	0	0	50	0	100	0	0	0	0	0	0	0	0	0
2012	Msy	75	DC	.	.	0	.	0	0	100	144	400	62	100	51	0	0	0	0	50	0
2012	Msy	84	DC	.	.	0	.	0	0	0	30	.	35	0	0	0	0	50	4	0	113
2012	Msy	5	DD	.	.	0	.	317	72	150	18540	0	0	50	4	100	0	0	0	0	37

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Msy	10	DD	.	.	0	.	50	0	200	119	100	0	0	0	0	0	800	0	0	0
2012	Msy	16	DD	.	.	0	.	0	3	0	0	100	13	0	0	0	83	0	0	0	0
2012	Msy	21	DD	.	.	0	.	0	0	50	0	0	0	0	0	50	0	36	0	0	0
2012	Msy	22	DD	.	.	0	.	.	.	0	237	0	.	.	.	0	0	.	.	200	32
2012	Msy	33	DD	.	.	0	.	250	.	0	0	0	0	0	0	0	0	.	.	0	0
2012	Msy	34	DD	.	.	0	.	.	.	100	0	100	0	300	144	50	0	87	0	400	8
2012	Msy	44	DD	.	.	0	.	0	9	0	0	150	0	0	0	0	51	50	0	200	0
2012	Msy	52	DD	.	.	0	.	0	0	100	0	.	.	337	5	100	0	300	0	0	0
2012	Msy	60	DD	.	.	0	.	.	.	50	0	50	0	.	.	0	0	950	0	700	66
2012	Msy	67	DD	.	.	0	.	250	100	50	0	0	1	100	0	50	0	0	0	0	0
2012	Msy	68	DD	.	.	0	.	0	18	150	595	0	0	100	131	0	0	50	0	0	86
2012	Msy	69	DD	.	.	0	.	.	.	50	0	0	0	.	.	0	0	100	0	800	58
2012	Msy	70	DD	.	.	0	.	100	74	0	0	300	6	.	.	50	0	.	.	800	114
2012	Msy	73	DD	.	.	0	.	200	0	0	0	150	0	0	0	0	1	550	102	0	48
2012	Msy	76	DD	.	.	0	.	350	0	50	0	0	0	182	36	247	0	92	0	250	0
2012	Msy	77	DD	.	.	0	.	129	6	100	0	0	0	0	0	50	0	0	0	50	1
2012	Msy	78	DD	.	.	0	.	250	73	400	0	150	0	0	0	0	0	850	0	250	73
2012	Msy	80	DD	.	.	0	.	.	.	0	0	150	21	250	0	0	0	1380	32	2050	73
2012	Msy	82	DD	.	.	0	.	200	0	0	0	50	0	0	0	65	15	50	0	250	3970
2012	Msy	3	DS	.	.	0	.	0	0	470	1960	0	0	0	0	0	0	0	0	0	0
2012	Msy	11	DS	.	.	0	.	.	.	0	0	0	0	0	1	300	17	0	0	0	2
2012	Msy	12	DS	.	.	0	.	0	0	1600	6	0	0	0	0	0	0	0	8	150	470
2012	Msy	13	DS	.	.	0	.	0	0	0	0	0	0	50	0	.	.	0	0	0	10
2012	Msy	15	DS	.	.	0	.	600	1	0	0	0	0	0	6	0	0	0	17	0	420
2012	Msy	20	DS	.	.	0	.	.	.	0	0	.	.	.	.	0	0	.	.	0	0
2012	Msy	24	DS	.	.	0	.	1200	0	1500	221	0	0	50	1	500	62	0	0	0	0
2012	Msy	26	DS	.	.	0	.	.	0	0	0	0	0	0	0	0	0	.	.	0	482

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Msy	28	DS	.	.	0	.	0	0	0	19	.	.	0	0	0	3	0	0	0	0
2012	Msy	37	DS	.	.	0	.	0	0	600	439	0	0	0	1	100	14	0	0	0	0
2012	Msy	38	DS	.	.	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	40	DS	.	.	0	.	0	0	200	204	0	0	.	.	0	0	200	83	1650	313
2012	Msy	42	DS	.	.	0	.	50	0	200	91	.	.	0	0	.	.	.	.	0	0
2012	Msy	45	DS	.	.	0	.	450	0	0	0	0	0	0	0	0	0	0	1	0	0
2012	Msy	48	DS	.	.	0	.	0	0	0	0	0	0	.	.	0	0	50	0	150	47
2012	Msy	50	DS	.	.	0	.	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	55	DS	.	.	0	.	900	0	0	0	0	0	100	0	0	0	50	44	300	677
2012	Msy	59	DS	.	.	0	.	100	0	1100	92	0	0	0	0	0	0	0	0	0	0
2012	Msy	71	DS	.	.	0	.	150	0	750	10	0	0	0	0	50	16	0	0	0	0
2012	Msy	81	DS	.	.	0	.	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	1	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2012	Msy	4	SP	.	.	0	0	0	0	0	0	0	0	.	.	0	0	0	0	0	0
2012	Msy	6	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	7	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0
2012	Msy	18	SP	.	.	0	0	0	0	.	.	0	0	0	0	0	0	0	0	0	0
2012	Msy	19	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	27	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	35	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	36	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	.	.	0	0
2012	Msy	41	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	43	SP	.	.	0	0	0	0	0	0	0	0	.	.	0	0	0	0	0	0
2012	Msy	46	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	49	SP	.	.	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2012	Msy	53	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	61	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2012	Msy	62	SP	.	.	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0
2012	Msy	64	SP	.	.	0	0	0	0	.	.	0	0	0	0	0	0	0	0	0	0
2012	Msy	72	SP	.	.	0	0	0	0	150	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	83	SP	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2012	Msy	85	SP	.	.	0	0	0	0	.	.	0	0	0	0	0	0	0	0	0	0
2013	Inv	239	DC	0	56	.	.	0	0	50	20	0	156	0	0	0	0	50	0	0	0
2013	Inv	251	DC	.	.	0	0	0	7	350	138	0	0	.	.	100	0	150	0	0	0
2013	Inv	262	DC	0	32	.	.	.	.	173	101	0	0	0	0	0	0	0	0	0	0
2013	Inv	270	DC	100	16	.	.	50	0	50	7	300	96	0	0	0	0	0	0	0	1
2013	Inv	281	DC	0	20	.	.	50	0	50	780	1300	360	0	0	0	0	50	0	300	1
2013	Inv	109	DC	.	.	0	0	.	.	.	.	.	.	0	0	0	0	100	0	100	0
2013	Inv	118	DC	100	80	0	0	50	5	400	450	850	808	0	0	0	0	50	0	0	0
2013	Inv	125	DC	50	20	0	0	.	.	.	.	250	196	0	0	.	.	.	.	.	.
2013	Inv	132	DC	150	23	.	.	0	1	0	79	300	32	0	0	0	0	0	0	0	0
2013	Inv	145	DC	150	44	.	.	.	.	0	22	.	.	50	0	0	0	0	0	0	0
2013	Inv	149	DC	.	.	.	.	.	.	.	.	50	0	0	0	0	0	0	0	0	0
2013	Inv	170	DC	50	153	0	0	0	0	50	32	150	44	0	0	0	0	0	0	0	0
2013	Inv	188	DC	50	121	.	.	0	0	305	612	600	6200	0	0	0	0	50	0	0	0
2013	Inv	202	DC	100	199	0	0	0	0	50	88	0	0	0	0	100	0	0	0	0	0
2013	Inv	217	DC	100	75	0	0	.	.	100	36	200	24	0	0	50	0	0	0	0	0
2013	Inv	223	DC	450	314	.	.	.	.	450	41	0	0	0	0	0	0	0	0	0	0
2013	Inv	270	DC	300	245	.	.	0	.	0	3	100	0	0	0	0	0	0	0	50	0
2013	Inv	277	DC	350	682	.	.	0	1	100	20	100	304	0	0	0	0	0	0	0	0
2013	Inv	282	DC	300	207	0	0	100	0	200	3	775	196	0	0	50	0	50	0	0	0
2013	Inv	212	DD	350	133	0	0	.	.	200	70	0	0	0	0	0	1	150	0	0	348
2013	Inv	230	DD	450	63	.	.	0	0	200	33	0	0	0	0	0	0	50	0	0	294
2013	Inv	242	DD	0	91	0	0	.	.	100	68	0	0	0	0	50	10	0	0	0	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2013	Inv	244	DD	493	.	0	0	0	3	200	251	.	.	50	0	0	0	0	0	0	4
2013	Inv	245	DD	150	111	.	.	0	0	350	514	0	0	0	0	0	0	0	0	50	0
2013	Inv	246	DD	300	41	.	.	50	0	350	61	0	16	0	0	200	0	700	118	0	0
2013	Inv	58	DD	150	7	0	0	0	0	100	168	0	0	.	.	50	0	0	0	0	284
2013	Inv	267	DD	0	6	.	.	.	.	200	120	0	0	0	0	50	3	.	.	0	0
2013	Inv	105	DD	150	31	.	.	0	0	0	251	0	0	0	0	0	0	0	0	0	0
2013	Inv	107	DD	250	60	0	0	0	0	150	83	0	0	0	0	0	0	250	84	250	1080
2013	Inv	111	DD	150	40	.	.	0	0	.	.	0	0	0	0	150	0	50	0	0	0
2013	Inv	122	DD	250	84	0	0	50	7	200	0	0	0	0	0	0	0	.	.	0	0
2013	Inv	131	DD	50	59	0	0	0	2	50	62	0	0	0	0	0	0	0	0	0	0
2013	Inv	136	DD	0	66	0	0	50	4	0	139	0	0	0	0	0	0	0	0	0	0
2013	Inv	152	DD	.	.	0	0	50	0	0	167	50	0	0	0	50	0	50	0	0	0
2013	Inv	234	DD	0	48	.	.	.	.	300	101	50	0	0	0	0	0	0	0	50	0
2013	Inv	239	DD	488	.	0	0	.	.	200	149	1194	176	700	71	0	1	50	0	0	116
2013	Inv	242	DD	100	199	.	.	0	0	150	54	0	8	0	0	300	0	.	.	0	0
2013	Inv	258	DD	200	458	.	.	50	0	.	.	.	.	.	.	.	.	.	.	0	0
2013	Inv	232	DS	150	23	0	0	.	.	0	86	0	16	0	.	0	0	50	0	0	0
2013	Inv	248	DS	.	.	.	.	0	1	20	95	100	0	0	0	0	0	0	0	0	0
2013	Inv	254	DS	50	11	0	0	0	26	150	122	0	0	0	0	0	0	0	0	0	0
2013	Inv	260	DS	.	.	.	.	50	11	50	105	150	172	50	0	0	0	50	0	50	0
2013	Inv	272	DS	300	224	.	.	0	3	0	196	100	96	.	.	.	.	0	0	0	0
2013	Inv	93	DS	100	0	.	.	100	2	200	22	0	0	0	0	0	0	50	0	0	0
2013	Inv	115	DS	50	7	0	0	50	0	100	138	0	0	.	.	0	0	.	.	0	0
2013	Inv	127	DS	50	60	0	0	50	50	250	303	0	0	0	0	0	0	50	0	0	0
2013	Inv	138	DS	.	.	0	0	.	.	100	154	0	0	50	1	50	0	50	0	0	1
2013	Inv	144	DS	100	99	.	.	50	2	250	56	0	0	0	0	0	0	0	0	100	0
2013	Inv	148	DS	0	347	.	.	.	.	50	1470	100	0	0	0	0	0	100	0	50	1

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2013	Inv	157	DS	0	13	0	0	100	0	150	8	0	0	0	0	0	0	0	34	0	0
2013	Inv	158	DS	100	300	.	.	0	2	150	43	200	352	150	9	0	0	0	0	0	0
2013	Inv	191	DS	150	18	.	.	.	.	200	2	0	0	0	0	0	0	0	0	0	0
2013	Inv	206	DS	100	87	.	.	0	0	50	33	0	0	0	0	0	0	0	0	0	0
2013	Inv	243	DS	0	45	0	0	0	3	50	61	0	0	0	0	50	0	100	0	0	0
2013	Inv	259	DS	50	58	.	.	0	0	0	4	0	0	0	0	0	0	50	0	0	0
2013	Inv	280	DS	50	45	.	.	.	.	0	0	0	28	0	0	0	0	0	0	0	0
2013	Inv	319	DS	550	326	.	.	0	0	50	32	450	164	0	0	0	0	0	0	0	0
2013	Inv	90	SP	100	28	0	0	0	0	.	.	0	0	.	.	0	0	.	.	0	0
2013	Inv	103	SP	55	26	0	0	0	0	.	.	0	0	0	0	.	.	0	0	0	0
2013	Inv	116	SP	100	45	.	.	.	.	0	0	0	0	.	.	.	.	0	0	0	0
2013	Inv	129	SP	0	43	.	.	.	.	.	.	0	0	0	0	.	.	.	.	0	0
2013	Inv	130	SP	0	9	.	.	.	.	0	0	.	.	.	.	.	.	.	.	0	0
2013	Inv	135	SP	150	25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0
2013	Inv	162	SP	400	.	0	0	0	0	250	0	0	0	.	.	0	0	.	.	0	0
2013	Inv	165	SP	0	199	0	0	.	.	.	.	0	0	.	.	.	.	0	0	0	0
2013	Inv	174	SP	150	36	.	.	.	.	0	0	0	0	.	.	0	0	.	.	0	0
2013	Inv	208	SP	50	190	0	0	.	.	0	0	0	0	.	.	0	0	0	0	0	0
2013	Inv	216	SP	0	19	.	.	.	.	.	.	0	4	.	.	.	.	.	.	0	0
2013	Inv	247	SP	50	301	.	.	.	.	.	.	0	0	.	.	.	.	.	.	0	0
2013	Inv	289	SP	1000	840	0	0	.	.	.	.	.	.	.	.	.	.	.	.	0	0
2013	Inv	294	SP	.	.	.	.	.	.	0	0	0	0	.	.	.	.	.	.	0	0
2013	Inv	297	SP	150	444	.	.	0	0	0	0	0	0	0	0	.	.	.	.	0	0
2013	Inv	301	SP	200	217	.	.	.	.	.	.	0	0	.	.	.	.	.	.	0	0
2013	Inv	324	SP	146	176	.	.	.	.	0	0	0	0	0	0	.	.	.	.	0	0
2013	Inv	271	SP	0	46	0	0	0	0	.	.	0	0	0	0	0	0	0	0	0	0
2013	Inv	275	SP	.	.	0	0	.	.	0	0	50	0	50	0	.	.	0	0	0	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2013	Msy	205	DC	400	12	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	212	DC	400	68	0	0	0	0	0	0	150	0	0	0	0	0	0	53	0	0
2013	Msy	218	DC	600	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	223	DC	250	127	0	0	0	0	50	0	0	0	0	0	0	0	200	4	0	0
2013	Msy	227	DC	.	.	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0
2013	Msy	233	DC	350	100	0	0	.	.	0	0	0	0	0	0	0	.	50	2	0	0
2013	Msy	237	DC	449	72	0	0	50	0	0	0	0	0	0	0	0	0	50	22	250	0
2013	Msy	242	DC	500	37	0	0	0	.	0	0	0	0	0	0	250	0	666	80	0	0
2013	Msy	243	DC	50	11	0	54	0	0	100	0	0	0	0	0	0	.	0	40	0	6
2013	Msy	245	DC	550	164	0	0	0	0	0	0	0	0	0	0	50	1	0	0	0	.
2013	Msy	250	DC	100	348	0	0	0	0	0	0	0	0	0	.	0	.	0	0	0	0
2013	Msy	252	DC	50	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	253	DC	550	54	.	.	0	0	0	0	0	0	50	0	0	0	50	0	0	0
2013	Msy	255	DC	300	708	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	256	DC	150	289	50	0	0	0	50	0	0	0	0	0	0	0	0	25	0	5
2013	Msy	260	DC	280	198	0	0	0	0	0	0	0	0	0	0	50	0	.	.	0	0
2013	Msy	268	DC	100	198	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	269	DC	600	5	0	0	0	0	0	0	0	0	0	0	50	0	0	1	0	0
2013	Msy	270	DC	0	5	0	0	0	0	0	0	0	0	.	.	0	0	0	.	0	0
2013	Msy	278	DC	350	311	0	0	0	0	0	31	0	0	0	0	0	0	150	48	0	0
2013	Msy	201	DD	750	44	0	0	0	0	0	0	0	0	0	0	0	45	150	82	50	0
2013	Msy	210	DD	200	176	0	0	.	.	0	0	0	.	.	.	0	95	0	31	0	0
2013	Msy	215	DD	0	127	0	0	0	0	0	.	0	0	0	0	0	20	50	1728	250	0
2013	Msy	216	DD	250	46	0	0	0	0	0	0	0	0	0	0	0	.	0	630	100	135
2013	Msy	220	DD	50	163	0	0	0	0	0	.	0	0	0	0	0	0	.	.	50	0
2013	Msy	221	DD	250	29	0	0	0	0	0	0	0	12	0	0	0	17	0	3260	0	0
2013	Msy	228	DD	100	115	0	0	0	0	0	0	0	0	0	0	50	55	50	107	100	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2013	Msy	229	DD	100	226	0	0	0	0	0	0	0	0	0	0	.	.	0	424	0	0
2013	Msy	231	DD	450	274	0	0	0	0	0	0	50	0	.	0	0	18	0	0	50	3
2013	Msy	236	DD	636	89	0	0	0	0	0	0	0	0	0	19	0	0	100	1	50	0
2013	Msy	244	DD	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	228	0	0
2013	Msy	249	DD	400	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
2013	Msy	251	DD	100	22	0	0	0	0	0	0	0	0	.	.	50	19	0	0	0	1
2013	Msy	254	DD	606	381	0	0	0	0	0	0	0	0	0	0	50	180	400	2845	100	1075
2013	Msy	257	DD	50	46	0	0	0	.	0	0	0	0	0	0	103	113	100	950	0	0
2013	Msy	258	DD	0	32	0	0	0	0	0	0	0	0	0	0	0	0	.	.	100	0
2013	Msy	264	DD	.	.	0	0	0	0	0	0	0	0	0	0	50	10	0	3	50	1
2013	Msy	271	DD	650	7	0	0	0	0	0	0	0	0	100	0	50	213	216	833	50	0
2013	Msy	281	DD	50	227	0	0	0	0	0	0	.	.	0	.	.	.	115	.	0	21
2013	Msy	202	DS	0	48	0	0	0	0	100	0	50	0	0	0	0	0	0	0	0	0
2013	Msy	203	DS	400	68	.	.	0	0	0	0	0	0	0	0	50	0	500	0	0	0
2013	Msy	206	DS	53	26	0	0	0	0	0	0	0	0	.	.	0	0	0	0	0	0
2013	Msy	219	DS	400	28	0	0	0	0	0	0	0	0	0	0	0	0	50	0	50	0
2013	Msy	224	DS	50	338	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	225	DS	150	149	0	0	0	0	0	0	.	.	0	0	0	0	0	0	0	0
2013	Msy	226	DS	196	35	0	0	0	0	.	0	0	0	0	.	0	0	.	.	0	0
2013	Msy	230	DS	100	227	.	.	0	0	0	0	0	0	0	0	0	0	50	0	100	0
2013	Msy	235	DS	284	222	.	.	0	0	0	0	0	0	500	0	0	0	0	0	0	0
2013	Msy	238	DS	400	62	0	0	0	0	0	0	0	0	0	0	0	0	102	0	0	0
2013	Msy	239	DS	256	103	.	.	0	0	0	0	0	0	0	0	.	.	0	0	0	0
2013	Msy	240	DS	450	63	.	.	0	0	0	0	50	0	150	0	345	0	0	0	0	0
2013	Msy	247	DS	100	124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	259	DS	200	153	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	265	DS	850	64	0	0	0	0	0	0	0	0	0	0	0	0	50	0	50	0

year	place	anim	treat	fec0	flc0	fec1	flc1	fec2	flc2	fec3	flc3	fec4	flc4	fec5	flc5	fec6	flc6	fec7	flc7	fec8	flc8
2013	Msy	267	DS	400	345	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	272	DS	0	227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	273	DS	300	43	0	0	0	0	0	0	.	.	.	.	0	.	0	0	50	0
2013	Msy	277	DS	250	77	0	0	0	0	0	0	50	0	0	0	0	0	0	0	50	0
2013	Msy	280	DS	250	8	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	204	SP	.	.	0	0	0	0	0	0	0	0	.	0	0	0	0	0	0	0
2013	Msy	207	SP	0	292	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0
2013	Msy	208	SP	150	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	209	SP	100	95	0	0	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	211	SP	300	30	0	0	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	213	SP	150	17	0	0	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	214	SP	450	231	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0
2013	Msy	222	SP	50	108	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0	0
2013	Msy	232	SP	150	167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	246	SP	0	57	0	0	.	.	0	1	0	0	0	0	0	0	0	0	0	0
2013	Msy	248	SP	200	88	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0
2013	Msy	261	SP	50	249	0	0	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	262	SP	100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	263	SP	300	6	.	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	266	SP	100	48	0	1	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	274	SP	350	95	0	0	.	.	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	275	SP	150	224	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	279	SP	250	413	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	282	SP	350	143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2013	Msy	283	SP	100	105	0	0	0	0	0	0	0	0	0	0	0	0	.	.	0	0

6.3. Data-worm burden

Anim= Identification number of the deer , Inv= AgReseach Invermay, Msy= Massey University Palmerston North, Treat= Treatment group, DD= deer own its own, DC= deer cross-grazing with cattle, DS= deer cross-grazing with sheep, SP= suppressively treated deer own its own, Set= set of tracers , if Set=1 first set of tracers euthanized, if Set= 2 second set of tracers euthanized, Oster= Ostertagia-type nematode, asy= *Spiculopteragia asymmetrica,* spi= *Spiculopteragia spiculoptera,* lep= *Ostertagia leptospicularis*, kol= Ostertagia kolchida, trif= *Teladorsagia trifurcate*, ostg= *Ostertagia ostertagi*, circ= *Teladorsagia circumcincta*, Haem= *Haemonchus contortus*, Trich Abo= *Trichostrongylus* spp. abomasum, axei= *Trichostrongylus axei*, ask= *Trichostrongylus askivali*, Coop= *Cooperia* spp., Trich SI= *Trichostrongylus* spp. from the small intestine, Oven= *Oesophagostomum venulosum.*

Anim	Place	Treat	Year	Set	Oster	asy	spi	lep	kol	trif	ostg	lyr	circ	Haem	Trich Abo	axei	ask	Coop	Trich SI	Oven
315	Inv	DC	2012	1	480	34	0	411	34	0	0	0	0	0	40	.	.	0	20	0
353	Inv	DC	2012	1	540	0	45	360	45	0	0	0	90	0	20	.	.	0	0	0
470	Inv	DC	2012	1	1140	0	0	903	190	0	0	0	48	0	0	.	.	0	60	10
332	Inv	DD	2012	1	2400	288	192	1440	480	0	0	0	0	0	20	0	20	0	0	0
335	Inv	DD	2012	1	1520	440	40	840	200	0	0	0	0	0	20	.	.	20	0	0
340	Inv	DD	2012	1	6840	1778	137	3557	1368	0	0	0	0	0	0	.	.	0	20	20
334	Inv	DS	2012	1	2880	0	0	1843	346	0	0	0	691	0	4480	4480	0	0	40	190
358	Inv	DS	2012	1	340	0	0	291	49	0	0	0	0	0	4080	4080	0	0	0	10
423	Inv	DS	2012	1	1080	0	0	836	174	0	0	0	35	0	3900	3900	0	0	220	310
355	Inv	SP	2012	1	1900	300	50	1350	200	0	0	0	0	0	60	60	0	0	0	0
429	Inv	SP	2012	1	300	0	0	300	0	0	0	0	0	0	260	260	0	0	0	0
442	Inv	SP	2012	1	620	0	0	455	41	0	0	0	124	0	180	180	0	0	100	0
368	Inv	DC	2012	2	67750	21172	0	45167	1411	0	0	0	0	0	600	0	600	0	0	70

Anim	Place	Treat	Year	Set	Oster	asy	spi	lep	kol	trif	ostg	lyr	circ	Haem	Trich Abo	axei	ask	Coop	Trich SI	Oven
451	Inv	DC	2012	2	41760	6682	1670	28397	5011	0	0	0	0	0	0	.	.	0	0	30
475	Inv	DC	2012	2	64750	13966	3809	45706	1270	0	0	0	0	0	0	.	.	0	0	0
317	Inv	DD	2012	2	8940	3755	179	4112	894	0	0	0	0	0	60	.	.	0	0	0
351	Inv	DD	2012	2	11100	2394	653	7182	871	0	0	0	0	0	0	.	.	0	0	0
440	Inv	DD	2012	2	22700	4006	0	17359	1335	0	0	0	0	0	0	.	.	0	0	0
347	Inv	DS	2012	2	4360	0	0	4011	349	0	0	0	0	0	1900	1900	0	0	0	0
444	Inv	DS	2012	2	5450	0	0	3633	681	0	0	0	1135	0	9550	9550	0	0	0	270
479	Inv	DS	2012	2	20700	0	0	10350	1656	2484	0	0	6210	0	31950	31950	0	0	260	0
323	Inv	SP	2012	2	1220	0	0	1109	74	0	0	0	37	0	20	20	0	0	0	0
350	Inv	SP	2012	2	1240	113	0	789	338	0	0	0	0	0	0	0	0	0	0	0
477	Inv	SP	2012	2	2160	47	0	1878	235	0	0	0	0	0	100	100	0	0	0	0
106	Inv	DC	2013	1	1540	55	770	220	110	0	0	0	385	0	380	380	0	0	0	370
126.2	Inv	DC	2013	1	1060	0	249	312	187	0	0	0	312	0	220	220	0	0	0	30
249	Inv	DC	2013	1	3040	0	1448	869	145	0	0	0	579	0	620	620	0	40	100	550
137.2	Inv	DD	2013	1	1180	62	745	124	0	62	0	0	186	0	120	120	0	0	0	70
306	Inv	DD	2013	1	700	0	467	117	0	0	0	0	117	0	160	160	0	0	0	80
311	Inv	DD	2013	1	720	48	384	288	0	0	0	0	0	0	60	60	0	0	0	30
96	Inv	DS	2013	1	2880	0	626	626	125	501	0	0	1002	0	200	200	0	0	0	100
141	Inv	DS	2013	1	1060	62	499	312	0	0	0	0	187	0	20	.	.	0	20	100
325	Inv	DS	2013	1	3140	0	1358	594	0	594	0	0	594	0	380	380	0	0	0	80
80	Inv	SP	2013	1	1540	0	963	385	0	0	0	0	193	0	180	180	0	0	0	110
147	Inv	SP	2013	1	500	0	318	182	0	0	0	0	0	0	40	40	0	0	0	20
166	Inv	SP	2013	1	920	66	460	263	66	66	0	0	0	0	20	.	.	0	0	10
41	Inv	DC	2013	2	1680	509	305	764	0	51	0	0	51	0	420	350	70	0	0	0
77	Inv	DC	2013	2	1260	164	219	712	110	0	0	0	55	0	120	120	0	0	0	20
201	Inv	DC	2013	2	600	240	120	120	40	40	0	0	40	0	340	340	0	0	0	0
123	Inv	DD	2013	2	7260	1320	660	3960	132	0	0	0	1188	0	360	216	144	0	0	0

Anim	Place	Treat	Year	Set	Oster	asy	spi	lep	kol	trif	ostg	lyr	circ	Haem	Trich Abo	axei	ask	Coop	Trich SI	Oven
124.2	Inv	DD	2013	2	11340	1512	1260	7560	504	0	0	0	504	0	1160	1160	0	0	0	10
161	Inv	DD	2013	2	2400	877	46	1431	0	0	0	0	46	0	80	80	0	0	0	0
59	Inv	DS	2013	2	2020	242	404	727	81	0	0	0	566	0	300	300	0	0	0	0
181	Inv	DS	2013	2	2000	375	292	333	0	250	0	0	750	0	120	120	0	0	0	20
323.2	Inv	DS	2013	2	1500	395	474	395	79	0	0	0	158	0	240	240	0	0	0	0
85	Inv	SP	2013	2	1120	523	149	373	75	0	0	0	0	0	0	.	.	0	0	70
172	Inv	SP	2013	2	740	269	67	404	0	0	0	0	0	0	20	.	.	0	0	0
244	Inv	SP	2013	2	1940	485	162	1078	54	0	0	0	162	0	100	100	0	0	60	480
111	Msy	DC	2012	1	160	.	.	.	.	.	.	.	.	20	0	.	.	0	0	30
115	Msy	DC	2012	1	140	.	.	.	.	.	0	0	.	0	20	.	.	0	0	20
130	Msy	DC	2012	1	120	.	.	.	.	.	0	0	.	0	0	.	.	0	0	10
116	Msy	DD	2012	1	6580	132	4211	2237	0	0	0	0	0	0	100	100	0	0	0	660
121	Msy	DD	2012	1	3780	79	2048	1654	0	0	0	0	0	20	20	.	.	0	0	0
124	Msy	DD	2012	1	5760	230	4147	1267	115	0	0	0	0	0	180	60	120	0	0	400
114	Msy	DS	2012	1	120	60	0	60	0	0	0	0	0	0	20	.	.	0	0	0
119	Msy	DS	2012	1	580	64	0	0	0	64	0	0	451	0	5440	5440	0	0	0	110
127	Msy	DS	2012	1	120	120	0	0	0	0	0	0	0	0	7840	7840	0	0	0	80
112	Msy	SP	2012	1	0	.	.	.	.	.	.	.	.	0	400	400	0	0	0	0
113	Msy	SP	2012	1	40	.	.	.	.	.	.	.	.	0	1680	1680	0	0	40	0
126	Msy	SP	2012	1	20	.	.	.	.	.	0	0	.	0	4280	4280	0	0	0	0
129	Msy	DC	2012	2	340	76	38	227	0	0	0	0	0	0	640	640	0	0	0	10
133	Msy	DC	2012	2	440	0	63	377	0	0	0	0	0	0	420	420	0	0	0	0
140	Msy	DC	2012	2	460	92	184	184	0	0	0	0	0	0	620	620	0	60	0	0
136	Msy	DD	2012	2	3820	153	382	3209	76	0	0	0	0	0	40	0	40	0	0	110
138	Msy	DD	2012	2	7840	143	1283	6129	285	0	0	0	0	0	20	.	.	0	0	0
139	Msy	DD	2012	2	11560	680	1133	8840	907	0	0	0	0	0	220	73	147	0	0	0
131	Msy	DS	2012	2	580	0	331	249	0	0	0	0	0	0	5560	5449	111	0	0	380

Anim	Place	Treat	Year	Set	Oster	asy	spi	lep	kol	trif	ostg	lyr	circ	Haem	Trich Abo	axei	ask	Coop	Trich SI	Oven
135	Msy	DS	2012	2	20	20	0	0	0	0	0	0	0	0	3020	3020	0	0	0	0
137	Msy	DS	2012	2	120	0	60	60	0	0	0	0	0	0	4260	4175	85	0	0	0
128	Msy	SP	2012	2	480	44	44	262	44	0	44	0	44	0	3800	3800	0	320	40	0
132	Msy	SP	2012	2	160	0	53	107	0	0	0	0	0	0	3760	3760	0	0	0	0
24	Msy	DC	2013	1	60	0	60	0	0	0	0	0	0	0	20	20	0	0	20	0
34	Msy	DC	2013	1	0	.	.	.	.	.	.	.	.	0	20	.	.	0	0	0
35	Msy	DC	2013	1	80	0	0	0	0	0	0	80	0	0	0	.	.	20	60	0
5	Msy	DD	2013	1	60	0	60	0	0	0	0	0	0	0	0	.	.	0	0	30
7	Msy	DD	2013	1	60	0	0	60	0	0	0	0	0	0	20	.	.	0	0	10
20	Msy	DD	2013	1	40	0	0	20	0	0	20	0	0	0	0	.	.	0	20	110
16	Msy	DS	2013	1	0	.	.	.	.	.	.	.	.	80	481	481	0	0	0	0
33	Msy	DS	2013	1	500	56	0	0	0	0	0	0	444	60	1322	1322	0	120	1320	80
39	Msy	DS	2013	1	0	.	.	.	.	.	.	.	.	0	180	180	0	0	980	0
3	Msy	SP	2013	1	60	0	60	0	0	0	0	0	0	0	20	.	.	0	0	0
8	Msy	SP	2013	1	0	.	.	.	.	.	.	.	.	0	0	.	.	0	0	0
12	Msy	SP	2013	1	0	.	.	.	.	.	.	.	.	0	0	.	.	0	0	0
11	Msy	DC	2013	2	1540	513	257	257	257	0	257	0	0	0	60	30	30	0	0	10
17	Msy	DC	2013	2	740	164	0	576	0	0		0	0	140	41	.	.	20	0	0
19	Msy	DC	2013	2	360	80	80	200	0	0		0	0	60	40	40	0	0	0	50
4	Msy	DD	2013	2	540	0	0	463	77	0	0	0	0	0	0	.	.	0	0	0
13	Msy	DD	2013	2	1000	500	125	250	125	0		0	0	0	0	.	.	20	0	0
28	Msy	DD	2013	2	340	68	136	68	68	0		0	0	0	0	.	.	0	0	0
1	Msy	DS	2013	2	140	0	0	140	0	0	0	0	0	60	682	682	0	20	40	20
23	Msy	DS	2013	2	1700	514	553	593	40	0		0	0	20	400	400	0	0	0	0
37	Msy	DS	2013	2	0	.	.	.	.	.	.	.	.	0	262	262	0	0	0	0
21	Msy	SP	2013	2	920	368	92	414	46	0		0	0	0	160	.	.	0	0	0
26	Msy	SP	2013	2	780	429	78	273	0	0		0	0	0	0	.	.	0	0	0

Anim	Place	Treat	Year	Set	Oster	asy	spi	lep	kol	trif	ostg	lyr	circ	Haem	Trich Abo	axei	ask	Coop	Trich SI	Oven
38	Msy	SP	2013	2	3220	1115	743	1300	0	0	0	0	62	0	20	20	0	0	0	0

Appendix 7 Standard Operating Procedures

7.1 SOP 1 Faecal Egg Count

Purpose
This SOP describes the procedure for performing faecal egg counts on animal faeces in order to calculate the number of nematode eggs per gram.

Responsibility
This SOP must be followed by all staff in the Parasitology Diagnostic Laboratory, IVABS.

Materials and Equipment

a) Workbook to record results
b) Scales to weigh faecal material (accuracy ± 0.1g)
c) Small sieve (tea strainer)
d) Small round bowl approx. 100ml capacity
e) Teaspoon
f) Pasteur pipette and rubber bulb
g) Saturated NaCl solution
h) Universal bottle (28ml capacity)
i) McMaster Egg counting slide
j) Microscope
k) Slide tray
l) Disposable rubber gloves
m) Paper towels
n) Hydrometer
o) Beaker for salt solution
p) Household salt
q) Mechanical mixer in salt container

Safety
For hygiene reasons always wear disposable gloves and laboratory coat.

Definitions
FEC = Faecal Egg Count; epg = Eggs per gram; NaCI = Sodium chloride (table salt);

s.g. = Specific gravity

Procedure

a) To make a saturated salt solution add salt to the blue plastic container until quarter full and then fill to near the top with hot water. Turn mixer on full. Test with a hydrometer the solution reads 1.2.s.g. More salt may be added as necessary.

b) Place the required number of sets of utensils on the bench (sets consist of a bowl, sieve and spoon).
c) Place the bowl, sieve and spoon on the scales and press tare then weigh out 2 grams of faeces.
d) Fill a universal bottle with saturated salt solution and pour it into the sieve. Work the faeces through the sieve using the teaspoon. Ensure the sieve is in the liquid whilst stirring. Discard the strainer and rinse any lumps off the spoon.
e) Place the required number of McMaster slides onto a slide tray.
f) Mix the contents of the bowl thoroughly with the teaspoon using a to-and-fro motion and at the same time remove a sample with a pipette. For diagnostic purposes the pipette can be rinsed with water between samples and reused.
g) Place the pipette at the opening of a chamber on the McMaster slide and quickly fill the chamber. Expel the remaining contents of the pipette back into the bowl.
h) Allow the slide to sit for 1-2 minutes to allow the eggs to float to the surface. This will not be necessary when doing 5-10 samples at one time.
i) Using the 10 X objective with 10 X eyepieces or 4 X objective with 15 X eyepieces, focus on the gridlines and air bubbles so that the eggs to be counted are on the same viewing level (never use the 40x objective).
j) Start at one corner of each counting grid and count eggs proceeding up and down the sections of the grid of both chambers. Count all eggs touching the top and left lines of each section but not the bottom or right hand lines. Multiply the total number of eggs by 50 to give the number of eggs per gram of faeces. This should be entered in the workbook.
k) Thoroughly clean all utensils under running water to remove all traces of faeces and replace in storage. Discard faecal samples in the bin for hazardous waste disposal.
l) If the sample weighs less than 2.0g record the weight in the workbook and use the formula: Eggs x 100 + weight to work out the epg.

Notes

Faecal egg counts are a useful diagnostic tool for ruminants.

The counting system relies on 2g faeces displacing 2ml fluid, which together with 28ml NaCI totals 30ml. The volume under each set of gridlines is 0.15ml (1cm x 1cm x 0.15cm) for a total of 0.3ml for a slide. This represents an aliquot of 0.01 of the original implying a multiplication factor of 100 X. As there were 2g of faeces each egg counted represents 50eggs/g.

History
The original SOP was written by SM Calder and W.E. Pomroy 25.11.98

Appendix

Manual of veterinary Parasitological Laboratory Techniques. Ministry of Agriculture. Fisheries & Food, UK, 1986

7.2 SOP 2 Strongylid Larval Culture and Identification

Purpose
This SOP details the procedure for preparing larval cultures and identifying infective larvae from cattle, goat, sheep, horses and deer faeces.

Responsibility
This SOP must be followed by all staff in the Parasitology Diagnostic Laboratory, IVABS.

Materials and Equipment

A) **Larval culture**
a) fine grade vermiculite
b) scoop
c) mortar and pestle or spatula
d) deionised water
e) glass jars and lids or plastic trays with glass lids
f) 27°C incubator
g) rubber gloves
h) marker pen, label or masking tape, for identification

B) **Baermann's apparatus**
a) Either 25 cm diameter glass funnels with rubber tubing and clamps attached or plastic bowls (15 cm diameter, 10 cm high with sloping slides)
b) kitchen sieve approximately 22 cm diameter with an aperture of 2 mm
c) clamp
d) stand for glass funnel
e) culture bottles
f) 10°C incubator
g) suction pump
h) measuring cylinder (1L or 2L)
i) Facial tissue or paper handkerchief.

C) **Identifying Larvae**
a) Slides
b) Coverslips
c) aqueous or Lugol's iodine
d) pipettes
e) bulb

f) multi-counter
g) eyepiece with micrometer

Safety
For hygiene reasons always wear disposable gloves and laboratory coat. If there is fungal growth in the cultures it is advisable to wear a protective face mask during mixing and recovery procedures

Procedure

a) Cleanliness is of utmost importance. Extreme awareness of not contaminating (introducing any foreign nematodes to the culture ie change gloves with each new sample).
b) Vermiculite container: Do not use scoop with dirty hands or gloves. Always remove gloves when needing more vermiculite in sample being cultured.
c) For a bulk culture, all the faecal material is mixed with Vermiculite and water with a spatula or gloved hands depending on which is more convenient, until a consistency is achieved whereby squeezing the culture results in excess moisture being expressed. If the faeces are pelletised they can be left to soak in deionised water until they are soft enough to breakup.
d) For diagnostic cultures a representative sample of faeces is taken from each animal in each group and mixed together (the groups being kept separate).
e) The culture is either placed loosely in glass jars until they are about half full, with the lid loosely applied, or placed in trays to a depth of about 4cm with a glass lid placed on top. The jars or trays must clearly show the date the egg counts were performed, the date the cultures were put up if different from the previous and identification of the sample.
f) The cultures are placed in a 25-27°C incubator for 10 days. Deionised water should be added if the cultures start to dry out. They should be kept moist not wet.
g) After 10 days the culture can be transferred to a Baermann's apparatus. A glass funnel is placed in a stand and a clamp is placed on the rubber tubing. A kitchen sieve is then lined with a single layer of facial tissue and then the faecal culture is added to a maximum depth of 3cm. More deionised water is then added to cover the faeces. Alternatively the sieve can be placed in a plastic bowl with deionised water instead of the glass funnel.
h) The culture is left in the Baermann's funnel for at least 6 hours, preferably overnight. The bottom 100-200ml is tapped off by opening the clamp and allowing the fluid to be collected in a measuring cylinder or beaker. If a bowl has been used the contents are gently siphoned off from the top of the solution until 2-3cm of fluid is left in the bowl. The sieve may be removed once the level of the solution is below the bottom of the sieve. If the sieve is removed before

siphoning the bowl must be left to stand for an hour before siphoning as the sediment will have been disturbed.

i) The solution is then transferred to a 1-2L measuring cylinder, filled up with deionised water and then left to sediment for 2 hours. The supernatant is then carefully removed from the top of the solution with a suction pump until 100-200ml remains. If the fluid is still dirty it should be re-sedimented until the supernatant is clear. This is most important for the storage of bulk cultures but not essential for diagnostic cultures.

j) Cultures are stored in plastic tissue culture bottles, on their side at a depth of approximately 1 cm in a 10°C incubator. The bottles are clearly labelled with the date and identification.

k) To identify the larvae they are concentrated by standing the culture bottle upright for half an hour. A subsample is removed from the bottom with a pipette and placed on a glass slide with a small drop of Lugol's or aqueous iodine to kill the larvae. Alternatively the slide can be flamed for approximately 3 seconds as this relaxes the larvae and causes them to straighten which aids measuring them. A coverslip is placed on top.

l) The slide is placed under the microscope and examined systematically. A total of 100 larvae are identified if present. The results are recorded in the workbook. Identification is made by reference to a standard text such as that mentioned in the Appendix.

History

This SOP is the original document and was written by SM Calder and W.E.Pomroy 25.11.98.

Appendix

Manual of Veterinary Parasitological Laboratory Techniques reference book 418. Pages 37 & 38.

7.3 SOP 3 Artificial infection of red deer with infective larvae (L3)

Purpose

The purpose of this SOP is to describe in detail the procedure for infecting deer with parasitic nematode infective stage larvae. All infections are applied orally.

Safety

Follow the guidelines given by Massey University regarding work clothes and large animals handling. When handling large animals including deer, make sure not to work alone. The use of crush, cradle or bale is necessary to restrain the deer for this procedure.

Materials and Equipment

a) Gloves
b) 2x Beaker (for fieldwork preferable plastic)
c) 4x Syringe (50ml).
d) 10cm rubber tube
e) Water
f) Larvae solution (adjust larvae concentration to 10-40ml solution per animal)

Procedure

a) Fill one beaker with larvae solution and toughly stir back and forth every time before filling syringe. Fill second beaker with water.
b) Fill syringe with appropriate volume of solution, get rid of any air bubbles and refill syringe to needed volume if necessary. Fill two syringes with water.
c) Secure animal head up with the crush and elevate the mouth.
d) Open the deer mouth and insert the tube until de stomach to ensure the larvae initially entered the rumen.
e) Insert tip of syringe with the larvae into the tube. Empty syringe slowly.
f) Repeat step e) with the two water filled syringe.

History

This SOP is a modified version for deer of the original document that was written Christian Sauermann (Sauermann 2014)

Tips

Beware – deer are very nervous and quite stressed. Do not get caught/squashed between the deer head and fixed objects!

7.4 SOP 4 Trickle artificial infection of red deer with infective stage larvae

Purpose

The purpose of this SOP is to describe in detail the procedure of infecting deer with parasitic nematode infective stage larvae. All infections are applied per os (orally).

Responsibility

This SOP must be followed by all staff.

Safety

Follow the guidelines given by Massey University regarding work cloth and large animals handling. When handling large animals including deer, make sure not to work alone. The use of crush, cradle or bale may be necessary to restrain the deer for this procedure.

Materials and Equipment

a) Gloves
b) Drench gun (20-30ml capacity, new/never been used with drench!!!)
c) 5 litre container for drench gun (new/never been used with drench!!!)
d) Sample tubes
e) Larvae in water

Procedure

a) Connect drench gun to container. Make sure the tube connecting the canister and the drench gun is secured firmly.
b) Gently rock the container whilst filling the drench gun. Make sure the lids are closed tightly before going to next step.
c) Prime drench gun.
d) Secure animal head with your arm or head bale and elevate the head.
e) Open animal mouth and dose the animal. The tip of the drench gun should be inserted into the cheek pouch or behind the dorsal prominence of the tongue. Before releasing animal or if more than one squirt has to be applied make sure the animal swallows the solution.
f) After dosing each animal gently rock the container to keep larvae from settling whilst refilling the drench gun.
g) After each pause (e.g. sampling, getting new animals in etc.) the solution has to be remixed by gently rocking the container. The fluid in the tube to the drench gun has to be replaced by squirting the drench gun 3-4 (solution can be put back into container).
h) Periodically take samples of the larvae solution: At specific times while infecting the animals fill a sample tube with a squirt of the drench gun. These samples should be analysed in the lab for checking larvae concentration and viability.

History

This SOP is a modified version for deer of the original document that was written Christian Sauermann (Sauermann 2014)

Tips

Beware – deer are very nervous and quite stressed. Do not get caught/squashed between the deer head and fixed objects!

7.5 SOP 5 Henriksen's modified Baermann's technique for detecting the presence of lungworm larvae in faeces

Purpose

This SOP describes the procedure for performing the Henriksen's modified Baerrnann's technique for the recovery of *Dictyocaulus* larvae from ruminant faeces.

Responsibility

This SOP must be followed by all staff in the Parasitology Diagnostic Laboratory, IVABS.

Materials/Equipment

a) Workbook to record results
b) Scales to weigh faecal material (accuracy ± 0.1g)
c) Conical styrene medicine measure of approx. 40ml volume (available from pharmacy stores)
d) Straightened paperclips or toothpicks
e) Cotton bandage, 10cm wide, cut in 7cm lengths
f) Counting slide
g) Compound microscope
h) Pasteur pipettes and bulb
i) Wash bottle
j) Spoon, spatula or popsicle stick.
k) Tap water at room temperature

Procedure

a) The piece of bandage is placed on the scales and tared.
b) 4.0g is weighed out onto the bandage. The bandage is wrapped around the faecal sample and then the paper clip is pushed through the gauze.
c) The sample is suspended by the paper clip over the medicine cylinder and lukewarm water is added until the sample is covered. Make sure all of the gauze is inside the cylinder and prevent the water from being siphoned out.
d) Leave the sample at room temperature overnight to allow the larvae to swim out of the faeces and fall by gravity to the depression in the bottom of the cylinder.
e) After standing overnight the supernatant is carefully removed leaving the larvae in the 0.5-lml of water at the bottom of the measure. Larvae can be counted by transferring the sediment to a McMaster slide and then focussing on the bottom of each chamber using a l0 X objective. If the sample is too dirty to count it can either be diluted with tap water or re-suspended and left to settle for 0.5- 1 hour.
f) To calculate the number of larvae per gram divide the total count by four.

Notes

Larvae identification may be necessary to ensure that the larvae seen are lungworm larvae and not other first stage larvae or free living nematodes.

History

This is the original document written by SM Calder and W.E. Pomroy on 25.11.98.

Appendix

- For pictures of lungworm larvae: Diagnosing Helminthiasis through coprological examination (Thienpont *et al.* 1979).
- Parasitology Practical Book l, WE Pomroy & WAG Charleston.

7.6 SOP 6 Diagnostic gastrointestinal worm counting

Purpose

This SOP details the procedure for the recovery of gastrointestinal nematodes in deer.

Responsibility

This SOP must be followed by all staff in the Parasitology Diagnostic Laboratory, IVABS

Materials/Equipment

a) string for tying off bowel segments
b) large trays
c) gut scissors
d) 500ml agee jars
e) stirring rod
f) 25ml & 50ml scoops
g) 2 X 10L buckets with 2L & 4L marked on them
h) disposable rubber gloves
i) Workbook to record results
j) multicounter
k) metal probe
l) petri dish with grid marked on it
m) black tray
n) stereomicroscope
o) beakers
p) wash bottle
q) teaspoon
r) 37.5µm sieve
s) marker pen, label or masking tape, for identification
t) magnifying lamp
u) water bath
v) 2L beaker
w) pepsin
x) hydrochloric acid
y) tin foil

Safety

Disposable gloves should be worn. A plastic apron maybe worn to guard against splashes

Procedure

Tie off the abomasum, small and large intestines with double ligatures and then separate. Remove all fat and connective tissue. If required freeze the individual organs.

A) Abomasum

a) Thawed the abomasum if frozen.
b) Open longitudinally over a bucket and then thoroughly wash the mucosa into the bucket with a jet of running water. Ensure no material is left under the folds.
c) The volume in the bucket should be made up to 2L or 4L and then mixed thoroughly with a to-and-fro motion with the stirring rod. While thoroughly stirring back and forth take out 5% aliquot by removing portions with a scoop (25 ml) and filling a measured container to the required volume. Repeat this step for a second 5% aliquot. Place one washed sample in a labelled jar in fridge for counting (can be stored up to a week if kept cool). Preserve second labelled sample as a reserve with formalin.
d) Wash and sieve the 5% counting sample using the 37.5µm sieve.
e) The washed abomasum was save for digestion at a later time.

B) Abomasal Digest.

a) The abomasa are digested to release inhibited larvae.
b) After washing the mucosa the organ is cut into small pieces and placed into a beaker containing a pepsin solution (600ml H20, 20g Pepsin and 10ml concentrated HCl). This is covered with tin foil and incubated for 2 hours in a water bath at 37°C.
c) After digestion the mucosa is washed and a 5% aliquot is taken and sieved in a 38 um sieve.
d) The aliquot are counted.

C) Small Intestine (S.I)

a) Thawed the S.I if frozen
b) Open the SI along its entire length and collect the contents in a l0L bucket. The mucosal surface is stripped by squeezing and massaging through the fingers under a gentle flow of water.
c) With water make up the volume to 4-l0L (deer).
d) Mixed thoroughly with a to-and-fro motion with the stirring rod. While thoroughly stirring back and forth take out 5% aliquot by removing portions with a scoop (25 or 50 ml) and filling a measured container to the required volume. Repeat this step for a second 5% aliquot. Place one washed sample in a labelled jar in fridge for counting (can be stored up to a week if kept cool). Preserve second labelled sample as a reserve with formalin.
e) Wash and sieve the 5% counting sample using the 37.5µm sieve.

D) Large intestine (L.I)

a) Thawed the L.I if frozen
b) Open the L.I longitudinally and collect the contents into a bucket. Wash the mucosa into the same bucket with a gentle stream of water.
c) With water make up the volume to 4-l0L (deer).
d) Mixed thoroughly with a to-and-fro motion with the stirring rod. While thoroughly stirring back and forth take out 10% aliquot by removing portions with a scoop (25 or 50 ml) and filling a measured container to the required volume. Repeat this step for a second 10% aliquot. Place one washed sample in a labelled jar in fridge for counting (can be stored up to a week if kept cool). Preserve second labelled sample as a reserve with formalin.

e) Wash and sieve the 10% counting sample using the 37.5µm sieve.

E) Counting and identifying parasites.

a) The aliquots from the abomasum and S.I are transferred in small portions to the counting dish generally in teaspoonful amounts depending upon how dirty the sample is.
b) The dish is placed on a stereomicroscope and examined systematically under 15-20 X magnification.
c) The genus and stage (adult or larvae) are recorded on a multi-counter.
d) If identification of a nematode is uncertain, the worm will be transferred to a microscope slide for identification under a compound microscoscope.
e) Large intestinal contents are transferred in small portions to a black tray and the contents are examined under a magnifying lamp.
f) The L.I nematodes are pulled out with a probe and genus and the stage (adult or larvae) are recorded on a multi-counter.

F) Species identification

a) In a microscope slide put drop of lactophenol (lactic acid, phenol crystals and distilled water) as clearing agent for nematode (Pritchard and Kruse 1982).
b) On the drop transfer the nematode for identification.
c) Put a cover slide.
d) Observed under a compound microscope.
e) Identification is made by comparison with a standard text (see Appendix).

G) Recording of results.

The number of worms found of each genus in the abomasum (5%), S.I (5%) and L.I (10%)

are multiplied by 20 or by 10 respectively, to give the total (100%) for each organ.

The results are written in work book.

History

This SOP slighty modified from the original that was written by W. E. Pomroy and SM Calder, 25.11.98.

Appendix

Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and

Food, UK, 1986.

7.7 SOP 7 Diagnostic *Dictyocaulus* spp. worm counting

Purpose

This SOP described the procedure for the recovery of lungworm nematodes in deer

Materials/Equipment

a) Workbook to record results
b) Marker pen, label or masking tape, for identification
c) Disposable rubber gloves
d) Haemostatic forceps
e) Gut scissors
f) Buckets of 20 litres and 10 litres
g) Trays
h) Sieve 37.5µm .
i) Pressure gauge
j) String rod
k) 500ml agee jars with name of the deer and the date
l) Small pottles with 70% alcohol for the worms.
m) Labels
n) Large trays
o) 25ml & 50ml scoops
p) Multicounter
q) Metal probe
r) Petri dish with grid marked on it
s) Stereomicroscope
t) Beakers
u) Wash bottle
v) Teaspoon

Safety

Disposable gloves should be worn. A plastic apron maybe worn to guard against splashes

Procedure

a) Thawed the lungs in a tray, lung should be attached to the heart and trachea
b) Then do an incision with the scissors on the right ventricle close to the pulmonary artery.
c) Insert the hose into the artery and ligate with a string rod (4 times, to ensure there is no return).
d) Use a bucket to receive the water, and put the bucket in a big container.
e) To hold the trachea to the bucket (20L) used to two haemostatic forceps. One in each side to keep it down.
f) Turn on water using a water pressure of 2.11 kg/cm^2. Ensure to have a haemostatic near in case there is a hole or fissure in the lung.

g) When the first 18 L are collected, turn off the water, and put the second bucket and collect 12 L. Make sure the buckets are labelled.
h) In the same way in 2 small buckets of 8 and 7 litres each. Please make sure the buckets are labelled. In total 45 L are collected.
i) In addition open the trachea until the bronchi division. It's easy to split the two lungs and then follow the bronchial tree with a fine blunt scissors. Once all the airways are exposed, they can be thoughtfully cleaned.
j) Then sieve the water obtain in each phase, using a 37.5µm size pore, and put the filtered residual in a labelled jar.

Counting and identifying parasites.

g) The 100% of the sieve water is put in small portions to the counting petri dish generally in teaspoonful amounts.
h) The petri dish is placed on a stereomicroscope and examined systematically under 15-20 X magnification.
i) The stage (adult or larvae) is recorded on a multi-counter.
j) If identification of a nematode is uncertain, the worm will be transferred to a microscope slide for identification under a compound microscoscope.
k) The total numbers of *Dictyocaulus* spp. are written in a work book.

7.8 SOP 8 PCR identification of parasitic nematode infective larvae

Purpose

This SOP describes the procedure for performing a multiplex PCR assay on infective larvae for the identification into species level.

Materials and Equipment

a) Workbook to record results
b) Scale.
c) 0.2ml thin-wall strip tubes
d) 8-strip domed Caps for 0.2 ml tubes.
e) 10ul Pipette Tips
f) 200ul Pipette Tips, Yellow
g) 1250ul Pipette Tips, Blue
h) Protective gear (includes laboratory coat and rubber gloves).
a) Pippetes (Pipetman Classic P10, P20 and P1000)
i) Pipette Eppendorf Multichannel Electronic 0.5ul-10ul
j) Universal bottles
k) Thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany)
l) Horizontal gel electrophoresis apparatus (including gel tray and combs).
m) Microwave oven and oven gloves.
n) Transilluminator (GelDoc™ XR, BIO-RAD Laboratories).

For lysis:

o) Fine platinum wire or eyelash (larvae picker)
p) Viagen DirectPCR (Tail) (Los Angeles, US) (cat#102-T)
q) Proteinase K recombinant (Roche, Basel, Switzerland)
r) Ultrapure water

For Multiplex PCR:

s) Platinum® Taq DNA Polymerase (Life Technologies, Carlsbad, USA) (cat#10342020)
t) dNTPs individuals 100mM (4 x 250uL) (Bioline, London, UK) (cat#BIO-39025)
u) Ultrapure water
v) Primers.

For electrophoresis:

w) Ultrapure Agarose (Invitrogen, Carlsbad, USA) (cat#16500100)
x) SYBR® Safe DNA Gel Stain (Invitrogen, Victoria, Australia) (cat#S33102)
y) 100 bp DNA ladder (Promega, Annandale, Australia) (cat# G2101)
z) TAE buffer*
aa) Loading buffer.

*** TAE Buffer includes Tris base, Acetic acid, 0.5 M EDTA, double-distilled water and KOH.**

Safety

For all the procedures used laboratory coat and disposable gloves, change gloves frequently.

Always wear oven gloves when heating the agarose solution.

Procedure

a) For larvae lysate:
a) For the preparation of the lysis solution, mix 1ml Viagen DirectPCR (Tail) (Los Angeles, USA) with 25ul Proteinase K (recombinant) (Roche, Basel, Switzerland).
b) Dispense 10µl aliquots of lysis solution into 0.2ml thin-wall strip tubes.
c) Pick an individual randomly-selected larvae from either 70% ETOH or H_2O into the above aliquots using a fine platinum wire or eyelash "larvae picker" making sure only one larva is in the tube.
d) Incubate tubes in PCR cycler overnight using following lysis programme: 55^{o}C 16 hrs, 90^{o}C 1hr, 4^{o}C 1min.
e) Freeze lysates until required.

PCR assay:

For the preparation of the multiplex mix for 96 larvae, mix:

- 700 µl 1.5 1.5mM MgCl2 Platinum Taq (Life Technologies, Carlsbad, USA) Master-mix*
- 40µl ITS2GF
- 40µl ITS2GR
- 30µl spp-specific primer 1
- 30µl spp-specific primer 2
- 30µl spp-specific primer 3
- 30µl spp-specific primer 4

That makes a total of 900 µl.

Select primers to give suitable spread of product sizes. Number of primers included can vary as long as final volume of the multiplex mix is maintained, this can be completed by adding the rest of the volume in water to ensure correct component concentrations.

*1.5mM MgCl2 Platinum Taq (Life Technologies, Carlsbad, USA) Master-mix:

- 515 µl H_20
- 100 µl 10xBuffer (included the Platinum® Taq DNA Polymerase)
- 30 µl 50mM $MgCl_2$ (included the Platinum® Taq DNA Polymerase)
- 50 µl 4mM dntp mix
- 5 µl Platinum Taq (included the Platinum® Taq DNA Polymerase)

That makes a total of 700 µl.

a) Put 9µl aliquots of the multiplex mix into 0.2ml thin-wall strip tubes.

b) Add 1µl of the template (i.e. larval lysate diluted up to 1:2 or 1:3 with sterile MQ water if necessary).
c) Spin down and mix briefly before running the reaction in the PCR thermal cycler.
d) Start the "Touchdown programme" in the thermal cycler.

Touchdown programme:

- Hot-start (Taq activation) – 95°C 8min
- Denature – 94°C 10sec
- Anneal – 60°C 15sec
- Extend – 72°C 30sec

} X 12 (annealing temp reducing 0.5°C/cycle)

- Denature – 94°C for 10sec
- Anneal – 54°C for 15sec
- Extend – 72°C for 30sec

} X 25

- Extra annealing–72°C for 7min
- Cool – 10°C for 1min

Visualization:

Electrophoresis:

a) Preparation of the 2.0 -3% agarose gel
b) Weight the ultrapure agarose (4- 6gr).
c) Insert into a high temperature resistant universal bottle.
d) To dissolve the agarose into TAE buffer (200 ml) heat in the microwave until bubbles appear (when hot used the oven gloves to hold).
e) Add 20µl Sybr safe of 10,000X SYBR Safe stain concentrate to the 200mls of the agarose gel solution.
f) Using the oven gloves put the solution on a large gel tray with 4 combs of 26 brackets each.
g) When the gel is set and cold (30-40 minutes) take the combs out.
h) Insert the gel tray in the Horizontal gel electrophoresis apparatus (with TAE buffer).
i) Mix the PCR template (10 µl) with the loading buffer (2 µl).
j) Load the mix (template+loading buffer) into the wells (5-8 µl).
k) Load at least one well in each line with the ladder (5 µl).
Start the electrophoresis (130 volt for 75 min).

After the electrophoresis to visualise PCR products, insert the gel in the transilluminator and photograph.

*50 X TAE Buffer Preparation protocol (Tris-Acetate-EDTA)
242 gm - Tris base
57.1 mL - Acetic Acid
100mL - 0.5 M EDTA (shake vigorously before use)
Add double-distilled water to 1 L and adjust pH to 8.5 using KOH.

History

This SOP is slightly modified version of the original document that was written in the Hopkirk (AgResearch) by Charlotte Bouchet.

7.9 SOP 9 Cloning "DNA genetic characterization" of deer parasitic nematodes

Purpose

This SOP describes the procedure for performing a DNA genetic characterization on deer parasitic nematodes.

Cloning "DNA genetic characterization" of deer parasitic nematodes

Materials and Equipment.

a) Workbook to record results
b) Scale.
c) 0.2ml thin-wall strip tubes
d) 8-strip domed Caps for 0.2 ml tubes.
e) 10ul Pipette Tips
f) 200ul Pipette Tips
g) 1250ul Pipette Tips
h) Protective gear (includes laboratory coat and rubber gloves).
i) Pippetes (Pipetman Classic P10, P20 and P1000)
j) Pipette Eppendorf Multichannel Electronic 0.5ul-10ul.
k) Universal bottles.
l) Thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany)
m) Horizontal gel electrophoresis apparatus (including gel tray and combs).
n) Microwave oven and oven gloves.
o) Transilluminator (GelDoc™ XR, BIO-RAD Laboratories).
p) Electroporator.
q) Nanodrop spectrophotometer
r) Water bath
s) Shaking incubator

For lysis:

t) Viagen DirectPCR (Tail) (Los Angeles, US) (cat#102-T)
u) Proteinase K recombinant (Roche, Basel, Switzerland)
v) Ultrapure water

For PCR amplification:

w) Platinum® Taq DNA Polymerase (Life Technologies, Carlsbad, USA) (cat#10342020)
x) dNTPs individuals 100mM (4 x 250uL) (Bioline, London, UK) (cat#BIO-39025)
y) Ultrapure water
z) Primers.

For Cloning:

aa) TOPO® TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, USA), with One Shot® TOP10 Electrocomp™ E. coli

(Includes PCR4-TOPO TA vector, electro-competent TOP10 cells, salt solution, S.O.C. medium)

bb) HPLC water.
cc) Ice
dd) Lysogeny broth media (LB) plates.
ee) Ampicillin.
ff) Qiagen" mini prep.

For electrophoresis:

gg) Ultrapure Agarose (Invitrogen, Carlsbad, USA) (cat#16500100)
hh) SYBR® Safe DNA Gel Stain (Invitrogen, Victoria, Australia) (cat#S33102)
ii) 100 bp DNA ladder (Promega, Annandale, Australia) (cat# G2101)
jj) TAE buffer*
kk) Loading buffer.

***** TAE Buffer includes Tris base, Acetic acid, 0.5 M EDTA, double-distilled water and KOH.

Safety

For all the procedures used laboratory coat and disposable gloves, change gloves frequently.
Always wear oven gloves when heating the agarose solution in the microwave.

Procedure

A. **For the nematode lysate:**

a) For the preparation of the lysis solution, mix 1ml Viagen DirectPCR (Tail) (Los Angeles, USA) with 30ul Proteinase K (recombinant) (Roche, Basel, Switzerland).
b) Dispense 30-100 µl (depending on nematode size) aliquots of lysis solution into 0.2ml thin-wall strip tubes.
c) Pick a nematode from H_2O into the above aliquot.
d) Incubate tubes in PCR cycler overnight using following lysis programme: 55^oC 16 hrs, 90^oC 1hr, 4^oC 1min.
e) Freeze lysates until required.

The lysate obtain above was diluted 1:50 or 1:100 with sterile distilled water and used as template in the PCR reaction to amplified the ITS-2 regions of rDNA.

For the amplification

a) The diluted lysate is mixed with the ITS-2 forward and reverse primers (5' TAGCTTCAGCGATGGATCGGT 3'and 5'CTTTTCCTCCGCTAAATGATATGC 3' respectively)

b) In addition mixed 2.5mM MgCl2 Platinum Taq Master-mix.
c) Spin down and mix briefly before running the reaction in the PCR thermal cycler.
d) Start the "programme" in the thermal cycler.

Programme:

- (1x) 95^{o}C 10min
- (35x) 94^{o}C 10sec, 55^{o}c 30 s, 72^{o}c 45 s.
- (1x) 72^{o}C for 10 min

After this process the result was run on agarose gel to check the presence of the correct product (follow SOP 5 "electrophoresis" and adjust for the size of the gel).

For the cloning

a) For the "ligation reaction" in a 0.2ml tube mix, 1 µl of the pcr product obtain in the previous amplification, 0.6 µl HPLC water, 0.4 µl diluted salt solution and 0.4 µl of the TOPO vector (Invitrogen, Carlsbad, USA). Incubate at room temperature for five minutes.
b) The ligated DNA was transformed by Electroporation into competent cells, for that. Cool 100µl electroporation cuvettes on ice
c) Thaw SOC and bring to room temperature.
d) Thaw electro-competent cells on ice; dilute each tube with 100 -150µl sterile water depending on how many reactions are needed
e) Add 45µl diluted electro-competent cells to each ligation reaction and transfer total volume immediately into an ice cold electroporation cuvette. Place on ice until electroporation
f) Set up electroporator – low range 200; high range 500; capacitance 25; voltage 1.5kv
g) Place cuvette into holder and press both pulse buttons simultaneously until buzzer sounds
h) Remove cuvette and quickly add 200µl SOC; mix briefly
i) Incubate cuvettes at 37^{o}C for ~1hr with occasional mixing
j) Plate out 50µl of transformed cells onto LB/amp plates and incubate at 37^{o}C overnight.
k) For "Re-plating selected colonies" Inspect the tranformant plates the following morning and transfer 5-10 good colonies from each onto a gridded LB/ampicillin plate using sterile pipette tips.
l) Incubate them at 37^{o}C for 8hr or until colonies are well grown.
m) For the "Clone verification" prepare PCR mastermix using components as per amplification reaction. The colony is mixed with the ITS-2 forward and reverse primers (5'TAGCTTCAGCGATGGATCGGT 3'and 5'CTTTTCCTCCGCTAAATGATATGC 3' respectively), mixed with 2.5mM MgCl2 Platinum Taq (Life Technologies, Carlsbad, USA) Master-mix. Use HPLC water instead of template. Aliquot 10µl to each tube
n) Add a touch of selected colony with a sterile 10µl pipette tip to transfer the template and mix by pumping the pipettor a few times.

o) Spin down and mix briefly before running the reaction in the PCR thermal cycler
p) Start the "programme" in the thermal cycler

Programme:

- (1x) 95^{o}C 10min
- (35x) 94^{o}C 10sec, 55^{o}c 30 s, 72^{o}c 45 s.
- (1x) 72^{o}C for 10 min

- Run PCR as per original reactions and run on gel to check for presence of correct product.

Plasmid purification.

Finally the Plasmid DNA was cut off using "Qiagen" mini prep.

Plasmid preparation:

- Set up cultures on selected colonies by transferring a small amount of transformant colony into 5ml LB/Kanamycin (Km) using a 10µl pipettor (5ml LB broth/5µl Km at 50mg/ml)
- Incubate ~15-16hr at 37^{o}C on rotary mixer
- Prepare plasmid DNA using Qiagen miniprep kit as per manufacturer's instructions.
- Estimate DNA concentration using the Nanodrop spectrophotometer.
- For the sequencing send samples to "Massey Genome Service", Palmerston North.

History

This SOP it is a slightly modified version of the original document that was written in the Hopkirk (AgResearch) by Charlotte Bouchet. In addition for most of the cloning procedure, it was followed the protocol by Invitrogen in the TOPO® TA Cloning® Kit for Sequencing. For the plasmid preparation, follow the instruction of the Qiagen miniprep kit.

Appendix 8 Publication and DRC 16

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Daniela Alejandra Tapia Escárate

Name/Title of Principal Supervisor: Prof William Ernest Pomroy

Name of Published Research Output and full reference:

Tapia-Escárate, D., Pomroy, W., Scott, I., Wilson, P., Lopez-Villalobos, N., 2015b, Establishment rate of sheep gastrointestinal nematodes in farmed red deer (Cervus elaphus). Vet. Parasitol. 209, 138-141

In which Chapter is the Published Work: 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 75%

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Planning and design of the experiment in agreement with supervisors. Field work and laboratory work in conjunction with technical support. Statistical analysis with guidance of supervisor. First draft of publication and corrections with assistance of supervisors.

Daniela Digitally signed by Daniela DN: c=NZ, cn=Daniela, o=Massey University, email=danielatapiaescarate@gmail.com Date: 2016.07.26 16:08:24 +12'00'

Candidate's Signature

26-7-2016

Date

William Pomroy Digitally signed by William Pomroy Date: 2016.07.26 16:52:10 +12'00'

Principal Supervisor's signature

26-7-2016

Date

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Short Communication

Establishment rate of sheep gastrointestinal nematodes in farmed red deer (*Cervus elaphus*)

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ABSTRACT

To investigate the establishment of sheep gastrointestinal nematodes (GIN) in red deer, five red deer and five sheep aged 5–6 months were challenged with a mixed burden of sheep GIN at a rate of 327L3/kg bodyweight. The LSmean (SE) establishment rates (%) for *Haemonchus contortus*, *Teladorsagia circumcincta*, *Cooperia curticei*, *Trichostrongylus* spp. and *Oesophagostomum* + *Chabertia* spp. were 18.6 (0.03), 35.5 (0.04), 30.7 (0.04), 74.9 (0.05), 19.9 (0.06), respectively in sheep and 10.5 (0.03), 1.0 (0.04), 0.1 (0.04), 1.0 (0.05), 4.8 (0.06) respectively, in deer. Establishment rates were significantly different ($p < 0.05$) between hosts for all genera. No *Trichostrongylus colubriformis* or *Trichostrongylus vitrinus* were seen in any deer but were present in all sheep. *Trichostrongylus axei* were seen in both hosts but there were relatively more which established in sheep than in deer ($p < 0.01$). No *Chabertia ovina* were seen in any deer but were present in four of five sheep in low numbers. The only species of *Oesophagostomum* seen in either host was *Oesophagostomum venulosum*. These results suggest that the sheep GIN most likely to infect red deer grazing the same pastures are *H. contortus*, *T. axei* and *O. venulosum*.

1. Introduction

As in other livestock production systems, parasites are an important clinical and economic problem in farmed deer (Audigé et al., 1998; Wilson, 2002). Whilst most focus has historically been on clinical disease caused by *Dictyocaulus* spp., gastrointestinal nematodes (GIN) may also be an issue for red deer (Audigé et al., 1998; Mason, 1977; Watson and Charleston, 1985). To help limit parasitism in deer there has been a move by deer farmers to use integrated management systems, particularly cross-grazing with other ruminants to restrict the number of deer-specific parasite larvae on pasture. However, very few studies have investigated the potential for cross-infection of GIN between deer and other ruminants. It is known that deer can be infected with some GIN of sheep including *Trichostrongylus axei*, *Haemonchus contortus*, *Oesophagostomum venulosum*, *Teladorsagia circumcincta*, *Trichostrongylus vitrinus*, *Nematodirus* and *Chabertia ovina* (McKenna, 2009). However, it is not clear how readily deer are infected with sheep nematodes. The aim of the present study was to determine the establishment rate of sheep GIN in young deer compared with sheep of the same age to help understand the potential risks associated with cross-grazing and susceptibility of deer to sheep GIN.

2. Materials and methods

Five male red deer calves (*Cervus elaphus*) and five Romney-cross ewe lambs (*Ovis aries*) raised on pasture which were born mid-November to early December 2011

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